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(54) Title: NON-STEROIDAL RADIOLABELED AGONIST/ANTAGONIST COMPOUNDS AND THEIR USE IN PROSTATE CANCER IMAGING

(57) Abstract

The present invention relates to non-steroidal radiolabeled androgen receptor ligands and their use in methods of imaging the prostate.
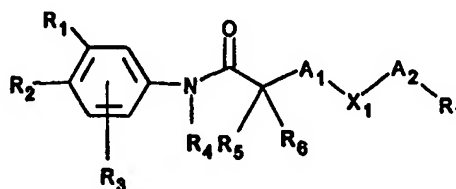
NON-STEROIDAL RADIOLABELED AGONIST/ANTAGONIST COMPOUNDS AND THEIR USE IN PROSTATE CANCER IMAGING

This application claims the benefit of U.S. Provisional Patent Application

5   Serial No. 60/049,376, which is hereby incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to non-steroidal radiolabeled androgen

10   receptor ligands and their use in imaging of prostate cancer.

BACKGROUND OF THE INVENTION

Throughout this application, various publications are referenced, many in

15   parenthesis. The disclosures of these publications in their entireties are hereby
incorporated by reference in this application.

Androgens are known to stimulate growth of the prostate and other
peripheral tissues, including primary or metastatic prostate tumor cells. Testosterone
("T") is the principal androgen secreted by the testes and is the primary circulating

20   androgen found in the plasma of males. The testes produce 95% of circulating plasma T,
while the remaining 5% is derived from the adrenals. In many target tissues, T is
converted by the enzyme 5α-reductase to the more potent androgen dihydrotestosterone
("DHT"). T and DHT then compete for binding to the androgen receptor ("AR") to exert
their influence on cell function. DHT has a four- to five-fold higher AR binding affinity

25   than does T and thus serves as the intracellular mediator for most actions of the hormone.
However, both androgens contribute to the overall androgenic effect. The high response
rate (i.e., 60 to 80%) to first line hormonal therapy and the presence of AR in both
primary and metastatic prostate tumor cells support the idea that the AR is an important
mediator of prostate cancer development and growth (Denis et al., "Prostatic Cancer: An

30   Overview," Acta. Oncol., 29:665-77 (1990) ("Denis"); McConnell, "Physiologic Basis of
Endocrine Therapy for Prostatic Cancer," Urol. Clin. N. Am., 18(1):1-13 (1991)).

Prostate cancer is the most frequently diagnosed non-skin cancer in
American men, accounting for approximately 27% of all cancer cases (Boring, Cancer

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Statistics 43:7-26 (1993)). Treatment for prostate cancer depends on the stage at which the cancer is found, and on the age and health status of the patient. As with other malignancies, accurate staging of prostate cancer is absolutely critical in selecting the most appropriate form of therapy. Clinically localized disease is potentially curable with

5 standard surgery and/or radiation therapy. However, no curative therapies exist for advanced disease. Existing diagnostic tests such as magnetic resonance imaging ("MRI"), computed tomographic scans ("CT"), and ultrasound ("US") lack both the specificity and sensitivity to substitute for exploratory surgery in staging malignant disease. Because of inadequate diagnostic studies, patients with what is presumed to be

10 surgically curable prostate cancer must submit to surgical staging to determine the presence or absence of lymph node metastases. Almost half of the men initially diagnosed with local disease are found to have tumors which have advanced to the periprostatic area or beyond at the time of surgery (Carter et al., In: "A Multidisciplinary Analysis of Controversies in the Management of Prostate Cancer," Coffey et al., eds.,

15 pp. 1-7, Plenum, New York). Thus, nearly one-third of all men diagnosed with prostate cancer (i.e., about 80,000 men per year) undergo surgery from which they are unlikely to benefit. Thus, non-invasive, more selective, and more accurate imaging tools for prostate cancer are needed.

Knowledge of the presence, location, and extent of disease aids in

20 selecting which patients are likely to benefit from radical surgery, radiotherapy, or androgen ablation. Single photon emission computed tomography ("SPECT") is a form of chemical imaging in which emissions from radiopharmaceuticals, labeled with gamma-emitting radionuclides (e.g. ^{99m}Tc, ^{67}Ga, ^{111}In, or ^{123}I) are used to create cross-sectional CT images of radioactivity distribution *in vivo*. Imaging of this type is typically

25 done with non-specific compounds (e.g. albumin or chelating agents like DTPA) complexed with the radionuclide. This, and similar methods, thus have the potential to improve the ability of CT and MRI to detect lymph nodes as well as bony and other visceral metastases. However, these methods are not selective.
Radioimmunoscintigraphy using monoclonal antibodies ("MoAbs") which recognize

30 prostate-specific proteins, including PSA and prostatic acid phosphatase ("PAP"), was developed as a means to specifically image the tumor, as opposed to the underlying host tissues. This approach has met with some success for these purposes (Dillman et al.,

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"Radioimmunodetection of Cancer With The Use of Indium-111-Labeled Monoclonal Antibodies," Natl. Cancer Inst. Monogr., 3:33 (1987); Vihko et al., "Immunoscintigraphic Evaluation of Lymph Node Involvement in Prostate Carcinoma," Prostate 11:51 (1987)). Prostascint is an IgG1 murine monoclonal antibody conjugated to the ^{111}In chelator

5   GYK-DTPA to form the immunoconjugate ^{111}In capromab pendetide. This antibody conjugate is reported to have a high degree of binding to all prostate cancers and mild binding to benign prostatic hypertrophic and normal prostate tissue. Preliminary data showed that this MoAb was able to detect disease foci of 5 mm or greater with a negative predictive value of 83% and positive predictive value of 50%, suggesting that

10   radioimaging is a promising technique for prognostication of prostate cancer (Babaian et al., "Radioimmunoscintigraphy of Pelvic Lymph Nodes With 111-Indium-Labeled Monoclonal Antibody CYT-356," J. Urol., 152:1952 (1994)). However, a major concern is that patients may develop human antimurine antibody ("HAMA") responses as a result of the murine origin of the antibody, resulting in adverse reactions and precluding the use

15   of subsequent antibody imaging (Babaian et al., "Radioimmunological Imaging of Metastatic Prostate Cancer With 111-Indium-Labeled Monoclonal Antibody PAY276," J. Urol., 137:439 (1987)).

     Chemical imaging represents a viable alternative to immunological methods. In this instance, the increase in imaging specificity is gained as the result of the

20   preferential distribution of a radiochemical into an anatomical region of interest. Wolf recently defined pharmacokinetic imaging as the measurement of the rate of change of a radiochemical in an anatomic space, or chemical imaging with the added dimension of time (Wolf, "Imaging Can Be Much More Than Pretty Pictures," Pharmaceut. Res., 12:1821-22 (1995)). This technique can be powerful, because it allows one to obtain

25   noninvasive measurements of the pharmacokinetics and pharmacodynamics of a radiolabeled compound at the target tissue site (Presant et al., "Association of Intratumoral Pharmacokinetics of Fluorouracil With Clinical Response," The Lancet, 343:1184-87 (1994); Dowell et al., "Pharmacokinetic Parameters of Cisplatin Can Be Estimated in Human Tumors by Noninvasive Measurements of the Biodistribution and

30   Targeting of [195m]Pt cisplatin," Proc. Am. Assoc. Cancer Res., 36:360 (1995)). Receptor-mediated chemical imaging has been used for the imaging of other endocrine tumors. [111]In pentetreotide (Octreoscan[®]) is used clinically for the imaging of

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somatostatin receptors present in neuroendocrine tumors. It has also been shown that 16

α-[^{18}F]fluoroestradiol and 21-[^{18}F]fluoro-16α-ethyl-19-norprogesterone can be used

with positron emission tomography ("PET") to provide clear images of both estrogen and

progesterone receptor-positive breast tumors (Mintun et al., "Positron Tomographic

5   Imaging of Estrogen Receptors in Human Breast Tumors," Radiology, 169:45 (1988);

McGuire et al., "Positron Tomographic Assessment of 16α-[^{18}F]-Fluoroestradiol Uptake

in Metastatic Breast Carcinoma," J. Nucl. Med., 32:1526 (1991); Pomper et al., "21-

[^{18}F]fluoro-16α-ethyl-19-norprogesterone: Synthesis and Target Tissue Selective

Uptake of a Progestin Receptor Based Radiotracer for Positron Emission Tomography,"

10   J. Med. Chem., 31:1360 (1988); Dehdashti et al., "Assessment of 21-[^{18}F]fluoro-16α-

ethyl-19-norprogesterone as a Positron-Emitting Radiopharmaceutical for Detection of

Progestin Receptors in Human Breast Carcinomas," J. Nucl. Med., 32:1532 (1991)). A

variety of steroidal androgens incorporating photon-emitting and positron-emitting

radionuclides have been synthesized and evaluated for their potential in imaging AR-

15   positive tumors of the prostate (Carlson et al., "A Comparative Study of the Selectivity

and Efficiency of Target Tissue Uptake of Five Tritium-labeled Androgens in the Rat,"

J. Steroid Biochem., 36:549 (1990) ("Carlson"); Brandes et al., "Fluorinated Androgens

and Progestins: Molecular Probes for Androgen and Progesterone Receptors with

Potential Use in Positron Emission Tomography," Molec. Pharmacol., 32:391 (1987)

20   ("Brandes"); Liu et al., "20-[^{18}F]fluoro-mibolerone, A Positron-Emitting Radiotracer For

Androgen Receptors: Synthesis and Tissue Distribution Studies," J. Nucl. Med., 32:81

(1991) ("Liu 1991"); Choe et al., "Synthesis of 11ß-[^{18}F]fluoro-5α-dihydrotestosterone

and 11ß-[^{18}F]fluoro-19-nor-5α-dihydrotestosterone: Preparation Via Halofluorination-

Reduction, Receptor Binding, and Tissue Distribution," J. Med. Chem., 38:816 (1995)

25   ("Choe"); Liu et al., "Synthesis of High Affinity Fluorine-Substituted Ligands for the

Androgen Receptor. Potential Agents for Imaging Prostatic Cancer By Positron Emission

Tomography," J. Med. Chem., 35:2113 (1992) ("Liu 1992"); Hoyte et al., "7α-methyl-17

α-(E-2'-[^{125}I] iodovinyl)-19-nortestosterone: A New Radioligand for the Detection of

Androgen Receptor," Steroids 58:13 (1993) ("Hoyte"); Ali et al., "Synthesis of 17α,

30   20E/Z)iodovinyl testosterone and 19-nortestosterone Derivatives as Potential

Radioligands for Androgen and Progesterone Receptors," J. Steroid Biochem. Mol. Biol.,

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49:15 (1994) ("Ali")). However, the majority of these compounds were not useful for AR-mediated imaging due to rapid metabolic cleavage of the radiolabel, low AR binding affinity, or inadequate specific activity. Carlson and Katzenellenbogen examined the target tissue selectivity of tritiated testosterone, dihydrotestosterone, 19-nortestosterone,

5   mibolerone (MIB), and methyltrienolone (R1881) in rats, concluding that compounds with AR binding affinities comparable to or greater than that of testosterone would be required to provide adequate target tissue uptake and target to non-target contrast for successful *in vivo* imaging of androgen target tissues (Carlson). MIB and R1881 demonstrated encouraging selectivity and target tissue uptake in animal models, most

10   likely due to their slower *in vivo* metabolic clearance as compared to the other androgens (Carlson; Brandes; Liu 1992; Choe; Liu 1991). Bonasera ("Preclinical Evaluation of fluorine-18-labeled Androgen Receptor Ligands in Baboons," J. Nucl. Med., 37:1009-15 (1996)) studied ^{18}F-labeled steroids using PET in baboons and is now studying 16ß-[^{18}F]fluoro-5α-dihydrotestosterone in men with metastatic prostate cancer.

15   Based on previous reports, the most important properties of radiolabeled androgens with respect to AR imaging appear to be: (i) the selectivity and affinity for AR binding; and (ii) the rate of *in vivo* metabolism (Carlson; Brandes; Liu 1992; Choe; Liu 1991; Hoyte; Ali). Androgenic steroids, like other steroids, are known to bind with other steroid receptors (Carlson; Dunn et al., "Transport of Steroid Hormones: Binding of 21

20   Endogenous Steroids to Both Testosterone-binding Globulin and Corticosteroid-binding Globulin in Human Plasma," J. Clin. Endrocrinol., 53:58 (1981) ("Dunn")). Binding of steroidal AR-imaging agents to progesterone and/or glucocorticoid receptors in the body contributes to their poor target site specificity for imaging. Radioactivity levels that remain in the blood or in non-target tissues are affected by the extent to which the agent

25   binds to high affinity, non-target proteins (Carlson; Choe; Ali). Further, the natural androgens (i.e., testosterone and dihydrotestosterone) are extensively bound to sex hormone-binding globulin ("SHBG"), a high affinity, low capacity binding plasma protein (Dunn). Not surprisingly, many of the synthetic androgens also bind to SHBG with high affinity (Carlson; Brandes; Liu 1991; Liu 1992; Choe; Hoyte; Ali). These high

30   affinity SHBG sites compete with the AR for specific binding of the radiolabeled ligand, and have precluded the use of a number of steroidal AR ligands for imaging. The metabolic fate and pharmacokinetics of AR-imaging agents are also important factors

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determining their usefulness for *in vivo* imaging (Carlson). Studies with estrogen and progestin imaging agents showed that *in vivo* target to nontarget uptake ratios correlated with the ratio of specific to non-specific binding *in vitro* (vanBrocklin et al., "16β-([^{18}F]fluoro)estrogens: Systematic Investigation of a New Series of Fluorine-18-Labeled

5   Estrogens as Potential Imaging Agents for Estrogen-Receptor-Positive Breast Tumors," J. Med. Chem., 35:1619 (1993)). However, it was recently found that this relationship did not hold for a series of androgen analogs (Choe). In the series of compounds therein, the compound with highest relative binding affinity ("RBA") and lowest non-specific binding had a poor target to nontarget tissue uptake ratio, while the compound with the lowest

10   RBA demonstrated the highest target to nontarget tissue ratio *in vivo*. As a whole, these data strongly suggest that *in vivo* metabolism is a major factor in determining the distribution profile *in vivo* (Carlson; Brandes; Choe; Ali).

The present invention is directed to overcoming these deficiencies.

15   **SUMMARY OF THE INVENTION**

The present invention relates to a radiolabeled non-steroidal compound having the formula:
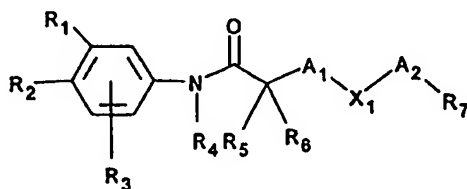
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where R_1, R_2 and R_3, are the same or different and are a radioactive or nonradioactive halogen, a nitro, a cyano, a carbamoyl, a hydrogen, a perfluoroalkyl, a haloalkylamido, an isothiocyanate, an azide, a diazocarbonyl, a substituted oxirane, or a β-

25   chloroethylamine;

R$_4$ is a hydrogen, an alkyl, or is joined to R$_5$;

R$_5$ is a radioactive or nonradioactive halogen, a hydrogen, a hydroxy, an alkyl, a haloalkyl, an alkoxy, an acyloxy, an amino, an alkylamino, a thio, a thioalkyl, or is joined to R$_4$;

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R_6 is a hydrogen, an alkyl, a halogen, or a haloalkyl;

A_1 and A_2 are the same or different, and each is a direct link or an alkylene;

X_1 is a radioactive or nonradioactive halogen, an oxygen, a sulfur, a

5   sulphinyl, a sulphonyl, an amino, an alkylimino, or an alkylene;

R_7 is a radioactive or nonradioactive halogen, a hydrogen, an alkoxy, a haloalkoxy, an acyloxy, a haloacyloxy, an aryloxy, a thioalkyl, a thioaryl, an alkylsulphinyl, an arylsulphinyl, an alkylsulphonyl, an arylsulphonyl, an amino, an alkylimino, an alkylamido group, a radioactive or nonradioactive haloalkylamido group,

10   or a phenyl optionally substituted with a radioactive or nonradioactive halogen, a nitro group, an alkyl, a radioactive or nonradioactive haloalkyl, a cyano, a hydroxyl, a carboxylic group, an amino, an alkylamino group, a dialkylamino group, a bisalkylamino group, a radioactive or nonradioactive haloalkylamino group, a radioactive or nonradioactive dihaloalkylamino group, a radioactive or nonradioactive

15   bishaloalkylamino group, an acylamido group, a diacylamido group, an alkylacylamido group, a radioactive or nonradioactive haloacylamido group, a radioactive or nonradioactive bis(haloacyl)imido group, or a radioactive or nonradioactive alkylhaloacylamido group.

The present invention also relates to a pharmaceutical composition which

20   includes a compound and a pharmaceutically suitable carrier.

The present invention further relates to a method of imaging for prostate cancer in a patient which includes contacting an androgen receptor with a radiolabeled non-steroidal compound under conditions effective to bind the radiolabeled non-steroidal compound to the androgen receptor of the patient and detecting the presence of any

25   radiolabeled non-steroidal compound bound to the androgen receptor.

The present invention also relates to a precursor compound having the formula as shown above, wherein R_1, R_2, and R_3 are the same or different, and each is a hydrogen, a halogen, a nitro, a cyano, a carbamoyl, a perfluoroalkyl, a haloalkylamido, an isothiocyanate, an azide, a diazocarbonyl, a substituted oxirane, a ß-chloroethylamine, a

30   diazonium salt, a triazene group, a tertiary alklyl group, an oxy group, an alkoxy group, a stannoalkyl group, a stannoaryl group, an unsubstituted or substituted boronic acid, an alkyl silane group, a pentaflourosilicate group, an alkylgermano group, a halomercury

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group, a trifluoroacetylthallate group, a thallium difluoride group, or other defined substituents;

R$_4$ is a hydrogen, an alkyl, or is joined to R$_5$;

R$_5$ is a hydrogen, a hydroxy, an alkyl, a haloalkyl, an alkoxy, an acyloxy,

5   an amino, an alkylamino, a thiol, a thioalkyl, a halogen, or is joined to R$_4$ or X$_1$;

R$_6$ is a hydrogen, an alkyl, a halogen, or a haloalkyl;

A$_1$ and A$_2$ are the same or different, and each is a direct link or alkylene;

X$_1$ is a halogen, an oxygen, a sulfur, a sulphinyl, a sulfonyl, an amino, or

an alkylimino, an alkylene, or is joined to R$_5$ directly, through an oxirane ring, through

10   an SO$_2$ group, or through an SO group;

R$_7$ is an aryl ring, substituted at different positions with a hydrogen, a

halogen, a diazonium salt, a triazene group, a tertiary alkyl amino group, a nitro group, an

oxy or an alkoxy group, an amino or an alkylamino group, a stannoalkyl group, a

stannoaryl group, an unsubstituted or a substituted boronic acid, an alkyl silane group, a

15   pentafluorosilicate group, an alkylgermano group, a halomercury group, a trifluoroacetyl

thallate group, or a thallium difluoride group.

Another aspect of the present invention relates to a method of producing

the radiolabeled non-steroidal compound which includes providing the precursor

compound, providing a radioactive compound, and reacting the precursor compound with

20   the radioactive compound under conditions effective to produce the radiolabeled non-

steroidal compound.

The compounds of the present invention have an AR binding affinity

several-fold greater than other nonsteriodal AR ligands and, accordingly, will bind and be

retained to the AR in primary and metastatic tumor cells. As such, they are useful in

25   imaging of prostate cancer and other tissues containing androgen receptors.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a bar graph that shows the binding affinities of compounds of

30   the present invention to AR.

Figure 2 is a graph that shows the HPLC purification of the R-isomer of an[125]Iodo-labeled compound of the present invention .

Figure 3 is series of graphs that show the retention of the R-isomer of an[125]Iodo-labeled compound of the present invention in the tissues of rats administered an
5   intravenous dose.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a radiolabeled non-steroidal compound
10   having the formula:
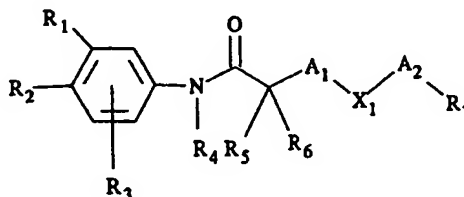
where R_1, R_2, and R_3 are the same or different and are a radioactive or
15   nonradioactive halogen, a nitro, a cyano, a carbamoyl, a hydrogen, a perfluoroalkyl, or a haloalkylamido;

R_4 is a hydrogen, an alkyl, or is joined to R_5;

R_5 is a radioactive or nonradioactive halogen, a hydrogen, a hydroxy, an alkyl, a haloalkyl, an alkoxy, an acyloxy, an amino, an alkylamino, a thio, a thioalkyl, or
20   is joined to R_4;

R_6 is a hydrogen, an alkyl, a halogen or a haloalkyl;

A_1 and A_2 is the same or different, each is direct link or an alkylene;

X_1 is a radioactive or nonradioactive halogen, an oxygen, a sulfur, a sulphinyl, a sulphonyl, an amino, an alkylimino, or an alkylene;
25   R_7 is a radioactive or nonradioactive halogen, a hydrogen, an alkoxy, a haloalkoxy, a acyloxy, a haloacyloxy, an aryloxy, a thioalkyl, a thioaryl, an alkylsulphinyl, an arylsulphinyl, an alkylsulphonyl, an arylsulphonyl, an amino, an alkylimino, an alkylamido group, a radioactive or nonradioactive haloalkylamido group,

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or a phenyl optionally substituted with a radioactive or nonradioactive halogen, a nitro group, an alkyl, a radioactive or nonradioactive haloalkyl, a cyano, a hydroxyl, a carboxylic group, an amino, an alkylamino group, a dialkylamino group, a bisalkylamino group, a radioactive or nonradioactive haloalkylamino group, a radioactive or

5   nonradioactive dihaloalkylamino group, a radioactive or nonradioactive bishaloalkylamino group, an acylamido group, a diacylamido group, an alkylacylamido group, a radioactive or nonradioactive haloacylamido group, a radioactive or nonradioactive bis(haloacyl)imido group, or a radioactive or nonradioactive alkylhaloacylamido group. The compound binds to an androgen receptor.

10   The subject invention is best understood through a discussion of receptors and signal transduction pathways. Cells in higher animals normally communicate by means of hundreds of kinds of extracellular signaling molecules, including proteins, small peptides, amino acids, nucleotides, steroids, retinoids, fatty acid derivatives, and even dissolved gases such as nitric oxide and carbon monoxide. These signaling molecules

15   relay a "signal" to another cell (a "target cell"), generally affecting a cellular function. As used herein, receptors for extracellular signaling molecules are collectively referred to as "cell signaling receptors".

            Many cell signaling receptors are transmembrane proteins on a cell surface; when they bind an extracellular signaling molecule (a ligand), they become

20   activated so as to generate a cascade of intracellular signals that alter the behavior of the cell. In contrast, in some cases, the receptors are inside the cell and the signaling ligand has to enter the cell to activate them; these signaling molecules therefore must be sufficiently small and hydrophobic to diffuse across the plasma membrane of the cell. As used herein, these receptors are collectively referred to as "intracellular cell signaling

25   receptors".

            Steroid hormones are one example of small hydrophobic molecules that diffuse directly across the plasma membrane of target cells and bind to intracellular cell signaling receptors. These receptors are structurally related and constitute the intracellular receptor superfamily (or steroid-hormone receptor superfamily). Steroid

30   hormone receptors include progesterone receptors, estrogen receptors, androgen receptors, glucocorticoid receptors, and mineralocorticoid receptors. The present invention is particularly directed to androgen receptors.

In addition to ligand binding to the receptors, the receptors can be blocked to prevent ligand binding. When a substance binds to a receptor, the three-dimensional structure of the substance fits into a space created by the three-dimensional structure of the receptor in a ball and socket configuration. The better the ball fits into the socket, the

5   more tightly it is held. This phenomenon is called affinity. If the affinity of a substance is greater than the original hormone, it will compete with the hormone and bind the binding site more frequently. Once bound, signals may be sent through the receptor into the cells, causing the cell to respond in some fashion. This is called activation. On activation, the activated receptor then directly regulates the transcription of specific

10   genes. But the substance and the receptor may have certain attributes, other than affinity, in order to activate the cell. Chemical bonds between atoms of the substance and the atoms of the receptors must form. In some cases, this leads to a slight change in the configuration of the receptor which is enough to begin the activation process (called signal transduction). As a result, substances can be made which bind receptors and

15   activate them (called receptor agonists) or inactivate them (called receptor antagonists).

The present invention is directed to compounds which are labeled antagonist or agonist compounds. Preferably, the compound of the present invention is a labeled non-steroidal ligand which binds the androgen receptor. Although it is preferable for the compounds to be antagonists, and, therefore, to inactivate the androgen receptor,

20   labeled agonist compounds may be used if desired.

The radiolabeled non-steroidal compounds of the present invention, which are useful in binding to the androgen receptor, are acylanilides having the formula:
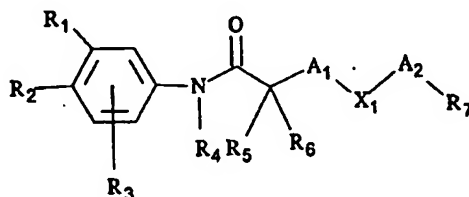
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wherein R_1, R_2 and R_3 are the same or different, and each is a radioactive or nonradioactive halogen, a nitro, a cyano, a carbamoyl, a hydrogen, a perfluoroalkyl, a haloalkylamido, an isothiocyanate, an azide, a diazocarbonyl, a substituted oxirane, or a β-chloroethylamine;

R_4 is a hydrogen, an alkyl, or is joined to R_5;

R_5 is a radioactive or nonradioactive halogen, a hydrogen, a hydroxy, an alkyl, a haloalkyl, an alkoxy, an acyloxy, an amino, an alkylamino, a thio, a thioalkyl, or is joined to R_4;

5　　　　R_6 is a hydrogen, an alkyl, a halogen, or a haloalkyl;

A_1 and A_2 are the same or different, and each is a direct link or an alkylene;

X_1 is a radioactive or nonradioactive halogen, an oxygen, a sulfur, a sulphinyl, a sulphonyl, an amino, an alkylimino, or an alkylene; and

10　　　　R_7 is a radioactive or nonradioactive halogen, a hydrogen, an alkoxy, a haloalkoxy, an acyloxy, a haloacyloxy, an aryloxy, a thioalkyl, a thioaryl, an alkylsulphinyl, an arylsulphinyl, an alkylsulphony, an arylsulphonyl, an amino, an alkylimino, an alkylamido group, a radioactive or nonradioactive haloalkylamido group, or a phenyl optionally substituted with a radioactive or nonradioactive halogen, a nitro

15　group, an alkyl, a radioactive or nonradioactive haloalkyl, a cyano, a hydroxyl, a carboxylic group, an amino, an alkylamino group, a dialkylamino group, a bisalkylamino group, a radioactive or nonradioactive haloalkylamino group, a radioactive or nonradioactive dihaloalkylamino group, a radioactive or nonradioactive bishaloalkylamino group, an acylamido group, a diacylamido group, an alkylacylamido

20　group, a radioactive or nonradioactive haloacylamido group, a radioactive or nonradioactive bis(haloacyl)imido group, or a radioactive or nonradioactive alkylhaloacylamido group.
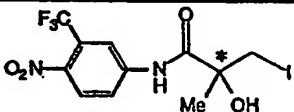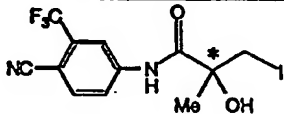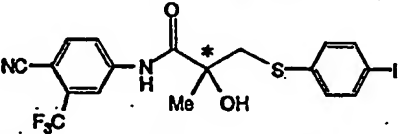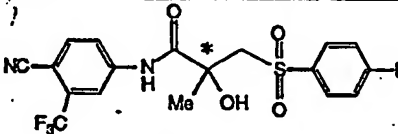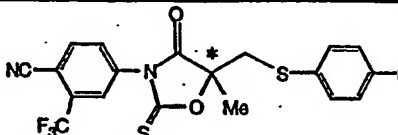
Compounds of the present invention may have affinity labels at either the R_7 or R_2 position, although compounds having the affinity label at other positions may

25　also be utilized.

A more preferred class of compounds have the above formula, wherein R_1 is CF_3, R_2 is CN or NO_2, R_3 is H, R_4 is H and R_5 is OH, or R_4 and R_5 are joined together as CSO; CSN, CSS, or CSC, R_6 is CH_3 or CF_3, A_1 is an alkylene or a direct link, A_2 is a direct link, X_1 is CH_2, S, or SO_2, and R_7 is a radioactive or nonradioactive halogen, or a

30　phenyl optionally substituted with a radioactive or nonradioactive halogen, a nitro group, an alkyl, a radioactive or nonradioactive haloalkyl, a cyano, a hydroxyl, a carboxylic group, an amino, an alkylamino group, a dialkylamino group, a bisalkylamino group, a

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radioactive or nonradioactive haloalkylamino group, a radioactive or nonradioactive

dihaloalkylamino group, a radioactive or nonradioactive bishaloalkylamino group, an

acylamido group, a diacylamido group, an alkylacylamido group, a radioactive or

nonradioactive haloacylamido group, a radioactive or nonradioactive bis(haloacyl)imido

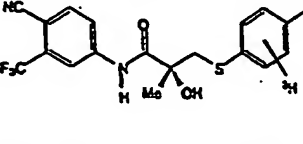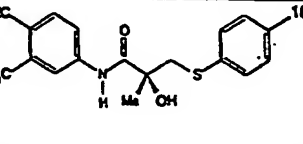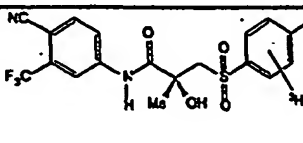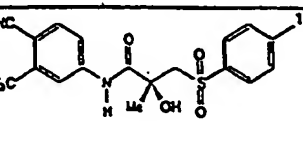5   group, or a radioactive or nonradioactive alkylhaloacylamido group.
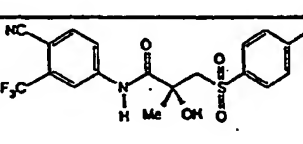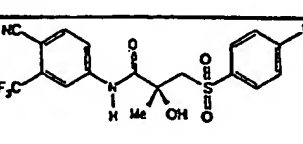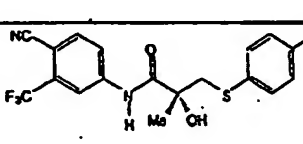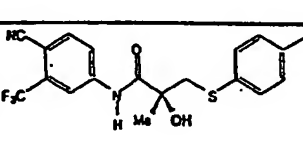
In particular, preferred compounds of the present invention are as shown

below in Tables 1 and 2.

Table 1

Compound	R-isomer	S-isomer
	Compound No.	Compound No.
	R-1	S-1
	R-2	S-2
	R-3	S-3
	R-4	S-4
	R-5	S-5

where Me is a methyl or trifluoromethyl group and I is radiolabeled (e.g., ^{125}I or ^{123}I).

Table 2

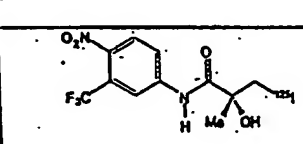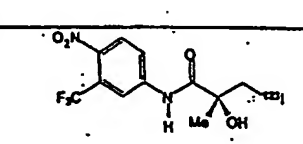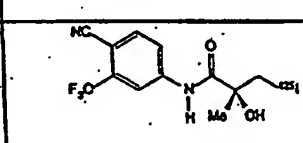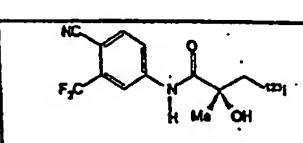| Iso-tope	Compound	Compound	Iso-tope
^3H			^{18}F
^3H			^{18}F
^{125}I			^{123}I
^{125}I			^{123}I
^{125}I			^{123}I
^{125}I			^{123}I

where Me is a methyl or trifluoromethyl group.

The high response rate to first line hormonal therapy and the presence of
AR in both primary and metastatic prostate tumor cells support the idea that AR is an
important mediator of prostate cancer development and growth. Accordingly, by binding
5　the compound of the present invention to the AR of a patient, therefore, the accurate
imaging of prostate tumors can be achieved. The present invention, therefore, is directed
to a method of imaging for prostate cancer in a patient. The method of imaging includes
contacting an androgen receptor with the radiolabeled non-steroidal compound under
conditions effective to bind the radiolabeled non-steroidal compound to the androgen
10　receptor, and detecting the presence of any radiolabeled non-steroidal compound bound

to the androgen receptor. Thus, an image of the prostate tumor cells with the radiolabeled compound bound to the tumor cells is obtained.

The compounds herein may be made up in any suitable form appropriate for the desired use; e.g., oral, parenteral (for example, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, or by application to mucous membranes, such as that of the nose, throat, and bronchial tubes, or by instillation into hollow organ walls or newly vascularized blood vessels) or topical administration. Suitable dosage forms for oral use include tablets, dispersible powders, granules, capsules, suspensions, syrups, and elixirs. The compounds may be administered alone or with suitable pharmaceutical diluents or carriers. Inert diluents and carriers for tablets include, for example, calcium carbonate, sodium carbonate, lactose, and talc. Tablets may also contain granulating and disintegrating agents such as starch and alginic acid, binding agents such as starch, gelatin, and acacia, and lubricating agents such as magnesium stearate, stearic acid, and talc. Tablets may be uncoated or may be coated by known techniques to delay disintegration and absorption. Inert diluents and carriers which may be used in capsules include, for example, calcium carbonate, calcium phosphate, and kaolin. Suspensions, syrups, and elixirs may contain conventional excipients, for example, methyl cellulose, tragacanth, sodium alginate; wetting agents, such as lecithin and polyoxyethylene stearate; and preservatives, e.g., ethyl-p-hydroxybenzoate.

Dosage forms suitable for parenteral administration include solutions, suspensions, dispersions, emulsions, and the like. They may also be manufactured in the form of sterile solid compositions which can be dissolved or suspended in sterile injectable medium immediately before use. They may contain suspending or dispersing agents known in the art.

It will be appreciated that the actual preferred amount of the compound to be administered according to the present invention will vary according to the particular compound, the particular composition formulated, and the mode of administration. Many factors that may modify the action of the inhibitor can be taken into account by those skilled in the art; e.g., body weight, diet, time of administration, route of administration, rate of excretion, condition of the subject, drug combinations, and reaction sensitivities and severities. Administration can be carried out continuously or periodically within the

maximum tolerated dose. Optimal administration rates for a given set of conditions can

be ascertained by those skilled in the art using conventional dosage administration tests.

Because the radiolabeled non-steroidal compounds of the present

invention bind to the AR and prostate cancer cells contain significantly higher levels of

5   AR than surrounding non-cancerous cells, it is possible to obtain an image of prostate

cancer cells to which the radiolabeled non-steroidal compounds have bound. Detection

of the radiolabeled non-steroidal compounds which have bound to the AR is performed

using, for example, single-photon emission computed tomography, positron emission

tomography, or other equivalent detection systems to obtain an image of the prostate

10   cancer. The detection occurs through the measurement of radioactive emissions by the

compounds of the present invention.

Another aspect of the present invention relates to methods of producing

the radiolabeled non-steroidal compounds of the present invention. The acylanilides of

the invention may be manufactured by any chemical process known to be suitable for the

15   manufacture of chemically-analogous compounds. Examples of suitable chemical

processes for manufacturing acylanilides are shown in U.S. Patent No. 4,636,505 to

Tucker, which is hereby incorporated by reference.

To synthesize the radiohalogenated AR ligands, a variety of precursors can

be used which have the formula shown below:

20

wherein R_1, R_2 and R_3, are the same or different, and each is a hydrogen,

a halogen, a nitro, a cyano, a carbamoyl, a perfluoroalkyl, a haloalkylamido, an

25   isothiocyanate, an azide, a diazocarbonyl, a substituted oxirane, a ß-chloroethylamine, a

diazonium salt, a triazene group, a tertiary alklyl group, an oxy group, an alkoxy group, a

stannoalkyl group, a stannoaryl group, an unsubstituted or substituted boronic acid, an

alkyl silane group, a pentaflourosilicate group, an alkylgermano group, a halomercury

group, a trifluoroacetylthallate group, a thallium difluoride group, or other defined

substituents;

R4 is a hydrogen, an alkyl, or is joined to R5;

R5 is a hydrogen, a hydroxy, an alkyl, a haloalkyl, an alkoxy, an acyloxy,

5   an amino, an alkylamino, a thiol, a thioalkyl, a halogen, or is joined to R4 or X1;

R6 is a hydrogen, an alkyl, a halogen, or a haloalkyl;

A1 and A2 are the same or different, and each is a direct link or alkylene;

X1 is a halogen, an oxygen, a sulfur, a sulphinyl, a sulfonyl, an amino, or

an alkylimino, an alkylene, or is joined to R5 directly, through an oxirane ring, through

10   an SO_2 group, or through an SO group;

R7 is an aryl ring, substituted at different positions with a hydrogen, a

halogen, a diazonium salt, a triazene group, a tertiary alkyl amino group, a nitro group, an

oxy or an alkoxy group, an amino or an alkylamino group, a stannoalkyl group (Sn-

Alk3), a stannoaryl group (Sn-Ar3), an unsubstituted or a substituted boronic acid, an

15   alkyl silane group (-SiR3), a pentafluorosilicate (-SiF5) group, an alkylgermano group

(-GeAlk2), a halomercury group (-HgHal), a trifluoroacetyl thallate group, or a thallium

difluoride group.

The precursors are reacted with suitable compounds under conditions

effective to produce the radiolabeled non-steroidal compounds of the present invention.

20   Generally, such suitable compounds contain a radioactive component (e.g., ^3H, ^{18}F, ^{125}I,

^{123}I, or other radiohalogens), which may be bound to the precursor in the step

immediately preceding formation of the compounds of the present invention, and may

include radioactive halide salts, hydrohalide acids, or elemental halides containing any

isotope suitable for imaging.

25   For example, the precursors used for radiohalogenation can be precursors

for aromatic nucleophilic substitution (i.e., aryl diazonium salts, aryl triazenes, tertiary

aromatic alkyl amines, nitroaryl precursors, aryl halides); precursors for aromatic

electrophilic substitution (i.e., aryl stannanes Ar-SnR3, aryl boranes Ar-BR2, aryl silanes

(Ar-SiR3), organopetnafluorosilicates (Ar-SiF5K2), aryl germanes Ar-GeR2, aryl

30   mercuriates Ar-HgCl, aryl thallates Ar-Tl(OCOCF3)2, Ar-H (direct halogenation *ortho-*

or *para-* to NH_2, or OH)); precursors for aliphatic nucleophilic substitution (i.e., triflates, mesylates and tosylates, trifluoroacetates, epoxides, cyclic sulfates, alcohols, alkyl halides); or precursors for aliphatic electrophilic substitution (i.e., Alk-BMe3).

Examples of preferred reactions are as follows:

5

Aryl diazonium salts in preparation of aromatic radiohalogenated
AR ligands

As shown in the scheme below, aromatic diazonium salts are useful

10 precursors for radiohalogenation (Knoechel et al., "Development of a Non-Carrier Method For 18f-Labeling," J. Labeled Comp. Radiopharm., 38:325-36 (1996); Muller et al., "Synthesis of [18F]NNC," Appl. Radiat. Isot., 46:323-28 (1995)). This so-called Sandmeyer reaction requires use of copper salts as a catalyst. The use of diazonium salts is described in Berridge et al., "Aromatic Fluorination With N.C.A. 18F-Fluoride: A

15 Comparative Study," J. Labeled Compd. Radiopharm., 22:687-94 (1985).
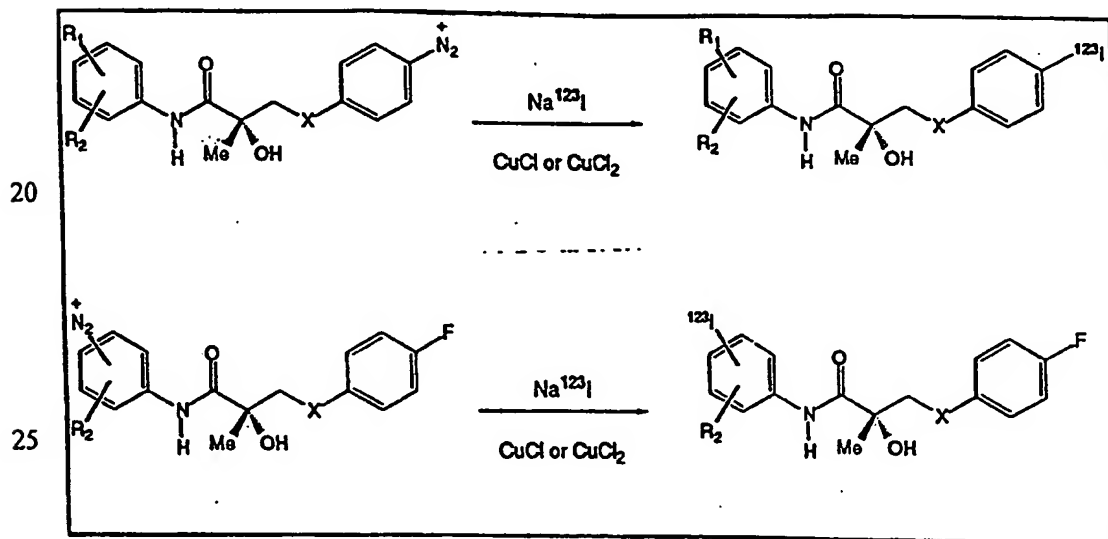
According to Ng et al., "Aromatic Fluorinations Suitable For Fluorine-18 Labeling of Estrogens," J. Org. Chem., 46:2520-28 (1981), decomposition of

30 tetraphenylborate diazonium salts gives fluorinated product. This reaction can be used for radiofluorination as illustrated below, where HF is a radiolabeled acid.
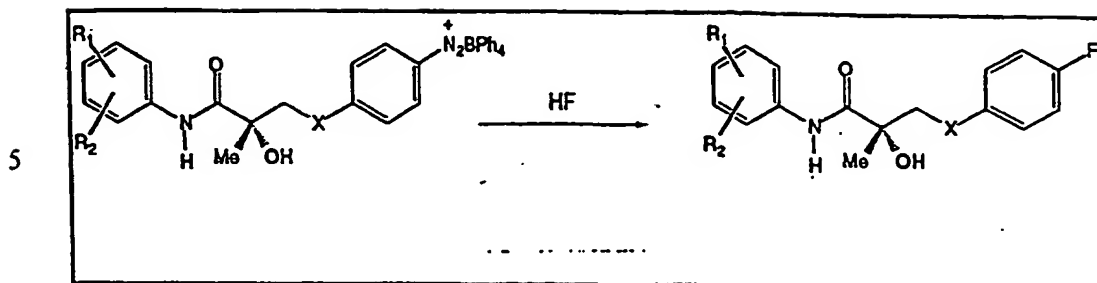
Aryl triazenes in preparation of aromatic radiohalogenated AR ligands

The scheme below shows the preparation of $[^{125}I]$- and $[^{123}I]$-radioiodinated or $[^{18}F]$-radiofluorinated compounds. In this scheme, acid-catalyzed nucleophilic substitution of triazene leads to the final radioiodinated ligands. This procedure is described in Counsell (Van Dort et al., "Radioiodinated P-Iodoclonidine: A High-Affinity Probe For The Alpha 2-Adrenergic Receptor," J. Med. Chem., 30:1241-44 (1987)).
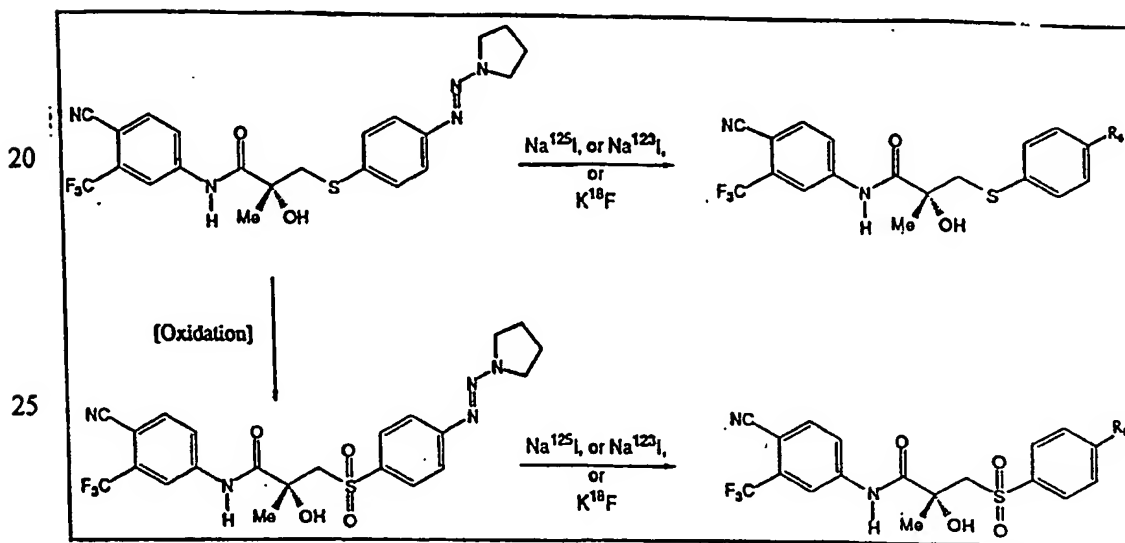
where R_8 is a phenyl substituent as set forth above, preferably a radiohalide.

Piperidiene triazenes also can be used for radiohalogenation.

Unfortunately, described yield of radiofluorination was very low (<4%) in the case of the reaction in DMSO at 180°C in the course of preparation of the analog of MPTP (Berridge

et al., "Design and Synthesis of 18F-Labeled Neurotoxic Analogs of MPTP," J. Med. Chem., 36:1284-90 (1993)) and in model reactions (Berridge et al., "Aromatic Fluorination with n.c.a. 18F-fluoride: A Comparative Study," J. Labeled Campd. Radiopharm., 22:687-94 (1985); Kilbourn et al., "Carrier-added and No-carrier-added

5   Syntheses of [18F]spiroperidol and [18F]haloperidol," Int. J. Appl. Radiat. Isot., 35:591-98 (1984)). Moderate yields (0-43%) of fluorinated products have been obtained by decomposition of piperidino triazenes in HF (Ng et al., "Aromatic Fluorinations Suitable for Fluorine-18 Labeling of Estrogens," J. Org. Chem., 46:2520-28 (1981)). The use of triazene derivatives for [^{18}F]radiofluorination, [^{78}Br, ^{77}Br]radiobromination, and

10   [^{125}I]radioiodination is described in U.S. Patent No. 4,431,627 to Eckelman et al. Fluorination using triazene as a precursor is described in Tewson et al., "Preparation of Fluorine-18 Aryl Fluorides: Piperidyl Triazenes as a Source of Diazonium Salts," J. Chem. Soc., Chem. Commun., 1149-50 (1979) and Berridge et al., "Aromatic Fluorination with n.c.a. 18F-fluoride: A Comparative Study," J. Labeled Campd.

15   Radiopharm., 22:687-94 (1985).

Tertiary aromatic alkyl amines in preparation of aromatic radiohalogenated AR ligands

20   Aromatic tertiary alkyl amines can be displaced with halogen. This method was used in the preparation of radiofluorinated GABA$_A$-gated chlorine ion exchange blocker (DMSO, 135°C, Kryptofix, NA^{125}I,) (Snyder et al., "Synthesis of Carbon-11, Fluorine-18, and Iodine-125-," J. Med. Chem., 38:2663-71 (1995)), and can be used for radiofluorination of AR ligands according to the scheme below.

25

30
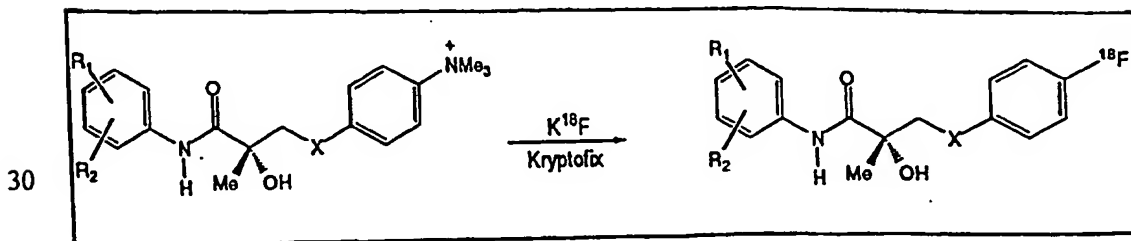
Nitroaryl precursors Ar-NO$_2$ in preparation of aromatic radiohalogenated AR ligands

Aromatic nitro compounds are used for substitution for fluorine. This

5   method was described in Kilbourn et al., "Fluorine-18 Labeling of Proteins," J. Nucl

Med., 28:462-70 (1987); Hwang et al., "A New Procedure for Labeling Alkylbenzenes

with [18F]fluoride," Int. J. Rad. Appl. Instrum.[A], 42:1043-47 (1991). Yields from good

(53-85%) to poor (2-9%) have been obtained with use of Bu$_4$NH^{18}F (TBA$\cdot$^{18}F) as a

carrier of fluorine-18 (Kilbourn et al., "Carrier-added and No-carrier-added Syntheses of

10   [18F]spiroperidol and [18F]haloperidol," Int. J. Appl. Radiat. Isot., 35:591-98 (1984)).

Nucleophilic displacement of aromatic nitro group has been widely studied in Attina et

al., "Labeled Aryl Fluorides from the Nucleophilic Displacement of Activated Nitro

Group by 18F-F-," J. Label. Comp. Radiopharm. 20:501-14 (1983). For

radiofluorination, the scheme below is appropriate.

15

20
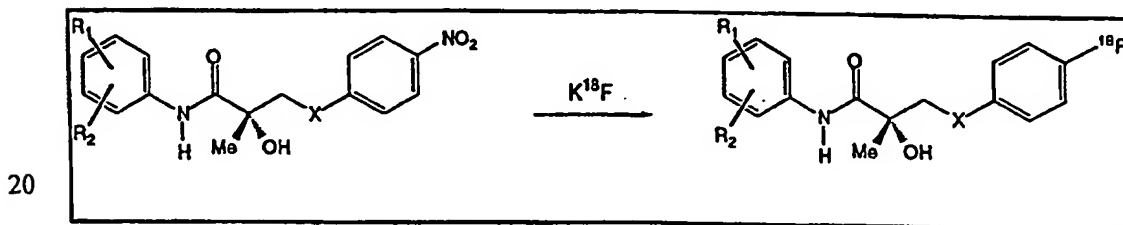
Aryl halides in preparation of aromatic radiohalogenated AR ligands. Halogen exchange reactions

25

Aromatic halogens can be exchanged with the same but radioactive

halogen atom (homoexchange), or another radioactive halogen (heteroexchange) atom at

elevated temperatures.

The exchange of iodine atom on ^{125}I has been described for 3,5-

30   diiodothirosine in an aqueous solution at 99°C in Breslav et al., In American Peptide

Symposium, 14th; Columbus, OH, (1995), and in the presence of ammonia sulphate

(NH)$_4$SO$_4$ and Na^{125}I at 145°C (Snyder et al., "Synthesis of Carbon-11, Fluorine-18,

and Iodine-125," J. Med. Chem., 38:2663-71 (1995)). For radiohalogenation, the scheme

below is appropriate.

- 22 -
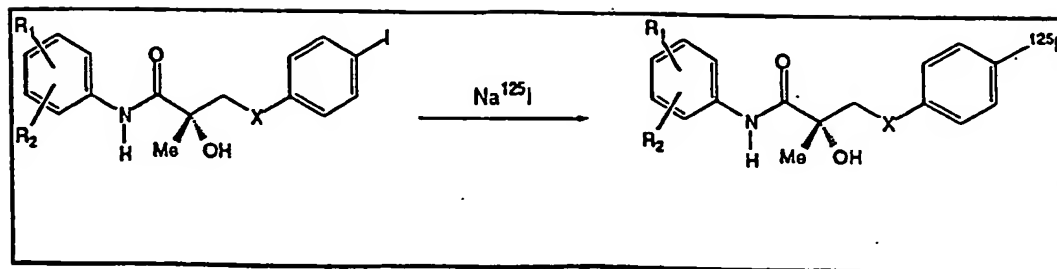
Exchange of iodine in preparation of radioiodinated lipids is described in

Meyer et al., "Potential Tumor or Organ-Imaging Agents. Radioiodinated Phospholipid

10   Ethers," J. Med. Chem., 32:2142-7 (1989); Counsell et al., "Tumor Visualization with a

Radioiodinated Phospholipid Ether," J. Nucl. Med. 31:332-36 (1990); Seevers et al.,

"Potential Tumor," J. Med. Chem., 25:1500-03 (1982). The same reaction is described in

DeGalan et al., "Iodine-125 Cholesteryl Iopanoate for Measuring Extent of

Artherosclerosis in Rabbits," J. Nucl. Med., 29:503-8 (1988); Counsell et al.,

15   "Lipoprotein Incorporation Enhances Radioiodinated Cholesteryl Ester Uptake into

Steroid Hormone-Secreting Tissues," J. Nucl. Med., 30:1088-94 (1989); Plotzke et al.,

"Selective Localization of Radioiodinated Alkylphosphocholine Derivatives in Tumors,"·

Int. J. Rad. Appl. Instrum. [B], 19:765-73 (1992).

The exchange of an iodine atom for ^{18}F has been described in Berridge et

20   al., "Aromatic Fluorination with n.c.a. 18F-fluoride: A Comparative Study," J. Labeled

Compd. Radiopharm., 22:687-94 (1985). The scheme below is appropriate for
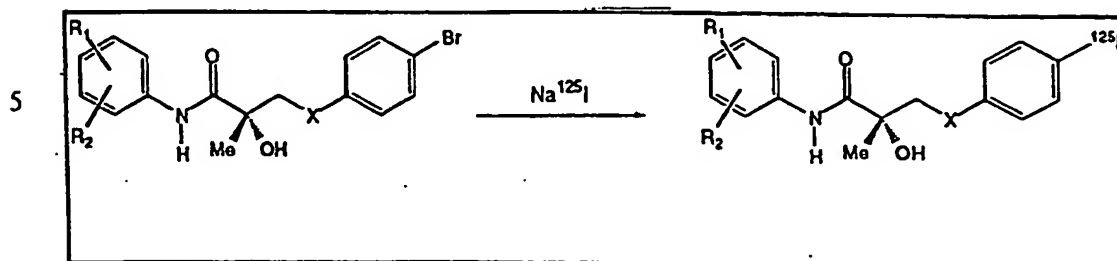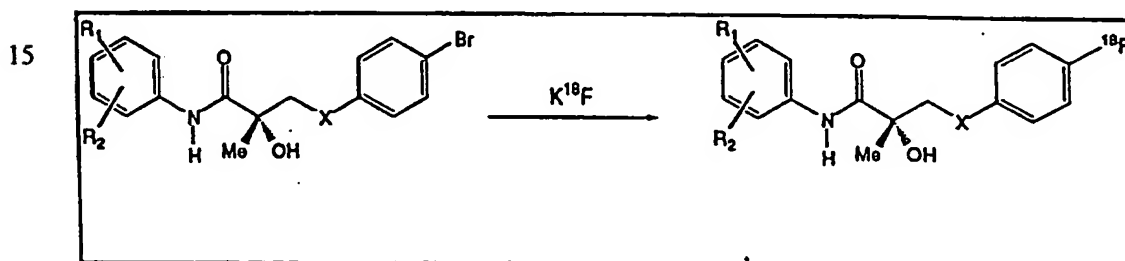
radiofluorination.
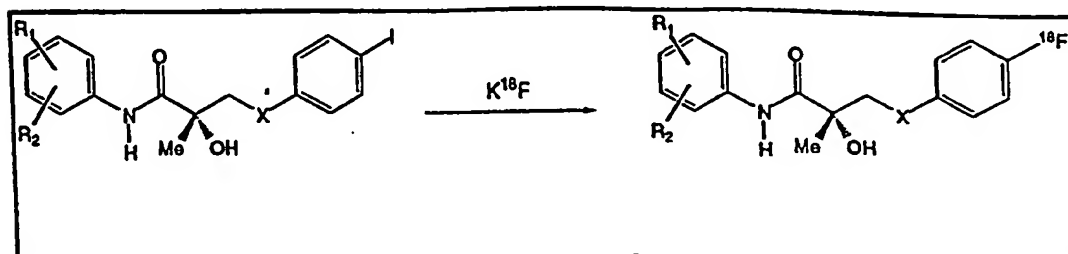
30   A single-step procedure for bromine-iodine exchange has been proposed

in Ceuster et al., "New Single-Step Radioiodination," J. Org. Chem., 60:8324-26 (1995).

- 23 -

A similar approach can lead to radioiodinated AR ligands of the present invention, as shown below.

5

$$Na^{125}I$$

10   The exchange of aromatic bromide for ^{18}F has been described in Berridge et al., "Aromatic Fluorination with n.c.a. 18F-fluoride: A Comparative Study," J. Labeled Compd. Radiopharm., 22:687-94 (1985). The scheme below is appropriate for radiofluorination.

15

$$K^{18}F$$

20

A chlorine-fluorine exchange as described in Kilbourn et al., "Carrier-added and No-carrier-added Syntheses of [18F]spiroperidol and [18F]haloperidol," Int. J. Appl. Radiat. Isot., 35:591-98 (1984) as shown below may be used for radiofluorination.

25

$$Bu_4NH^{18}F$$
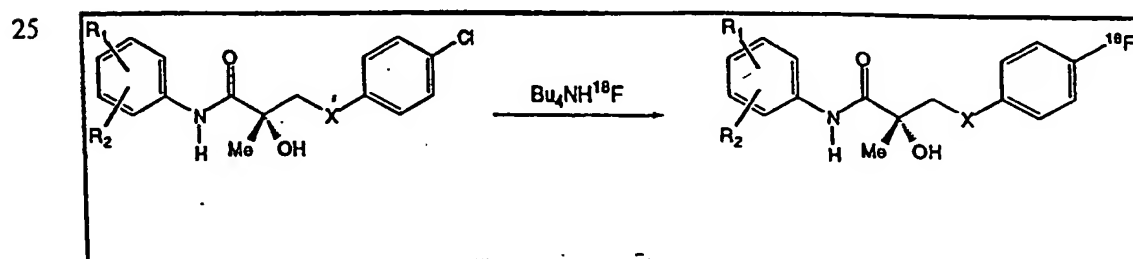
30

Fluorine-fluorine exchange as described by Kilbourn et al., "Carrier-added and No-carrier-added Syntheses of [18F]spiroperidol and [18F]haloperidol," Int. J. Appl.

- 24 -

Radiat. Isot., 35:591-98 (1984) as shown below may be used to prepare radiofluorinated product. This reaction was also described in Attina et al., "Labeled Aryl Fluorides from the Nucleophilic Displacement of Activated Nitro Group by 18F-F-," J. Label. Comp. Radiopharm., 20:501-14 (1983).
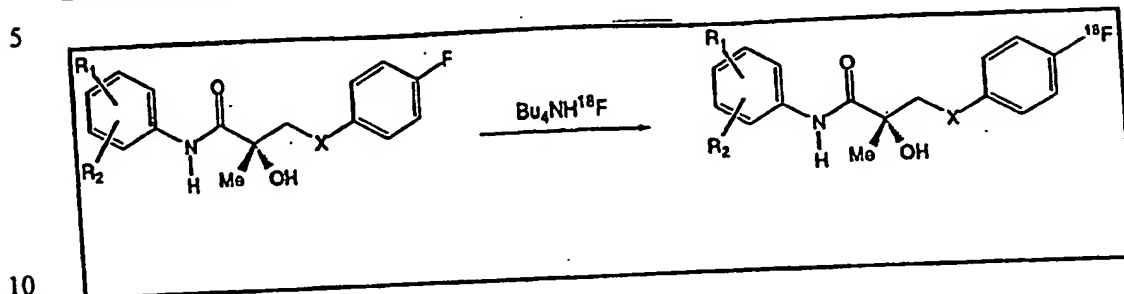
5

10

Aryl stannanes in preparation of aromatic radiohalogenated AR ligands

15   The scheme below shows the use of tributyltin precursor for the synthesis of radioiodinated AR ligands. (John et al., "Synthesis and Pharmacological," Cancer Res., 55:3022-27 (1995); ((Mais et al., "Novel Synthesis and Biochemical Properties," J. Med. Chem., 34:1511-14 (1991)) Gallant et al., In ACS Meeting. 209th; ACS: Anaheim, CA April 2-6, 1995 pp. MEDI-171 (1995)); Hodson et al., "Regiospecific Synthesis,"

20   Perkin Trans. 1, 2965-68 (1995); Wardell et al., "Reactions of Aromatic Sulfenyl Compounds with Organotin Compounds," J. Organomet. Chem., 78:395-404 (1974)).

The advantages of these precursors in the incorporation of radiohalogens are their easy preparation and chemical stability. However, they are highly toxic (Kabalka, "The Synthesis of Radiolabeled Compounds Via Organometallic

25   Intermediates," Tetrahedron, 45:6601-21 (1989)).
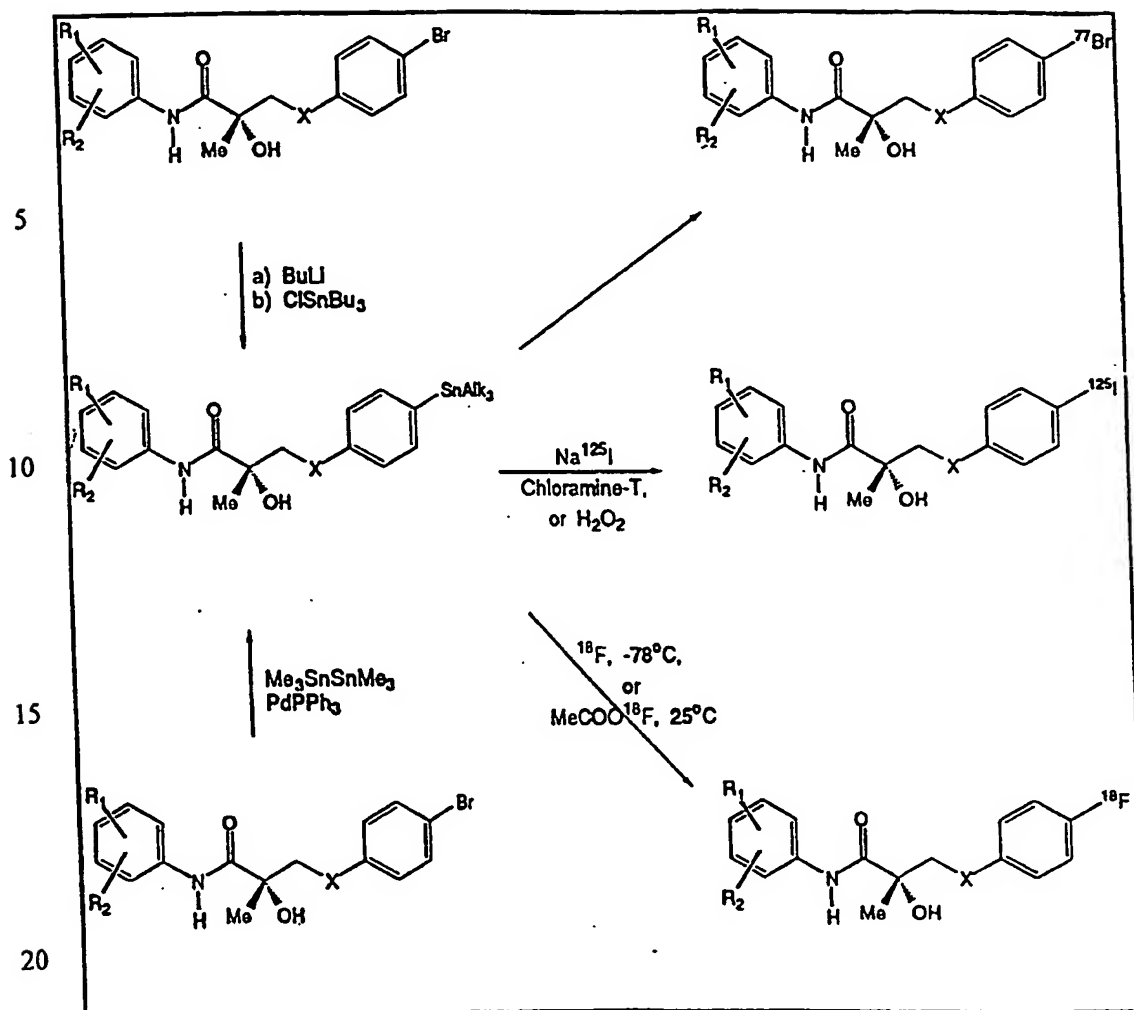
As shown below, triphenyltin derivatives can be reacted with elemental fluorine for radiofluorination according to a procedure described in Berridge "Chemistry of Fluorine-18 Radiopharmaceuticals," Appl. Radiat. Isot., 37:685-93 (1986).
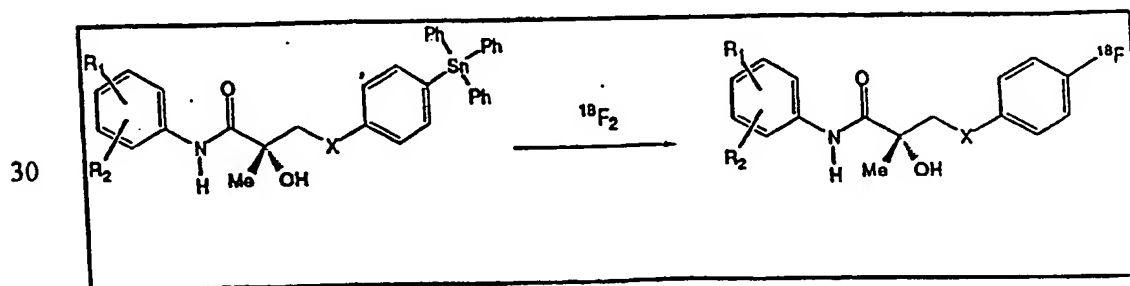
<u>Aryl boranes in preparation of aromatic radiohalogenated AR ligands</u>

The iodination of an aromatic ring can be achieved via the direct reaction
of arylboronic acid with radiolabeled sodium iodide in the presence of an oxidizing agent
(Kabalka, "The Synthesis of Radiolabeled Compounds Via Organometallic
Intermediates," Tetrahedron, 45:6601-21 (1989). Similar precursors can be used for
[18F]-radiobromination, [77Br]-radiochlorination, and [34mCl]-radiochlorination.

<u>Aryl silanes (Ar-SiR₃), and organopentafluorosilicates (Ar-SiF₅K₂)
in preparation of aromatic radiohalogenated AR ligands</u>

Arylsilanes shown below are valuable precursors for radiohalogenation
under a variety of conditions and forms of halogen used (Kabalka, "The Synthesis of
Radiolabeled Compounds Via Organometallic Intermediates," Tetrahedron, 45:6601-21
(1989)).

- 27 -

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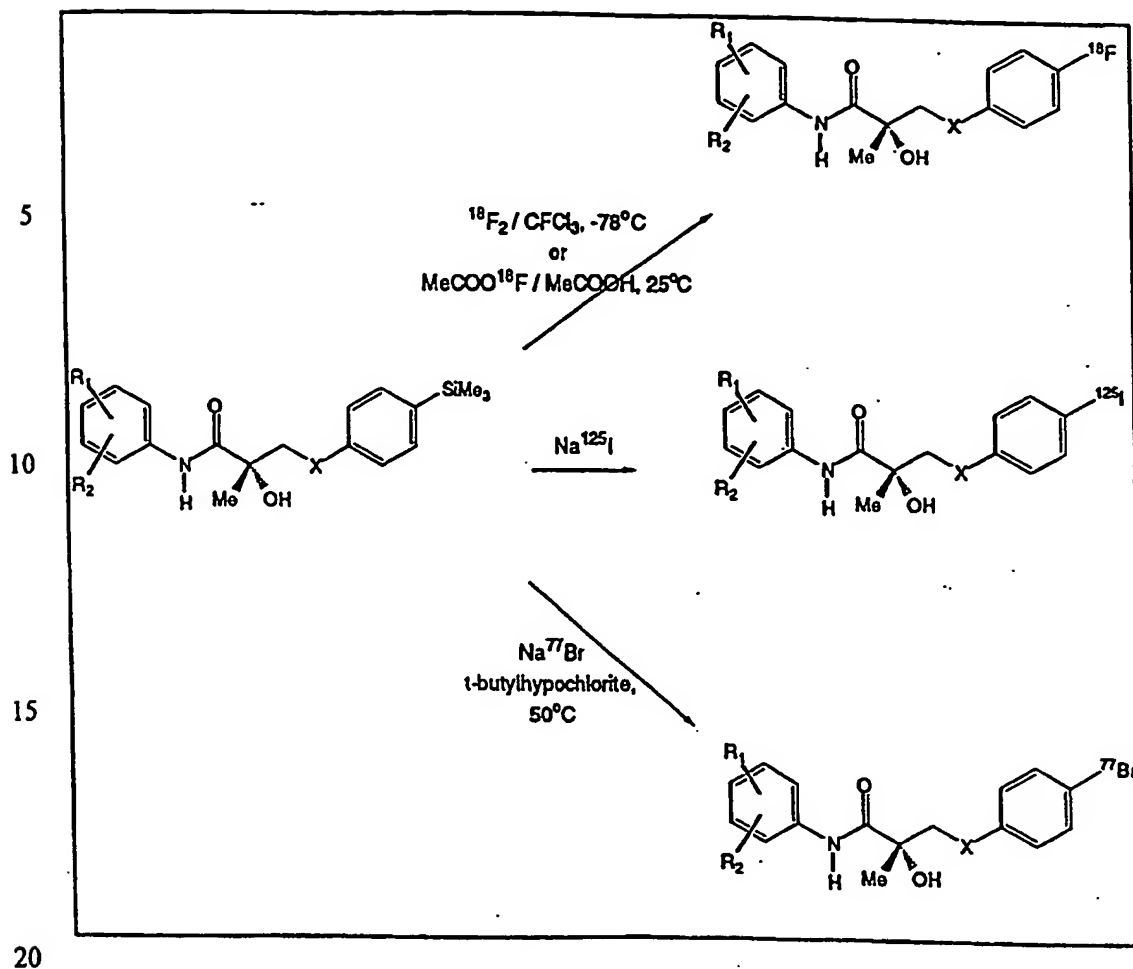
Organopentafluorosilicates are air and moisture stable precursors of radiohalogenated compounds (Kabalka, "The Synthesis of Radiolabeled Compounds Via Organometallic Intermediates," Tetrahedron, 45:6601-21 (1989)). They can be used for
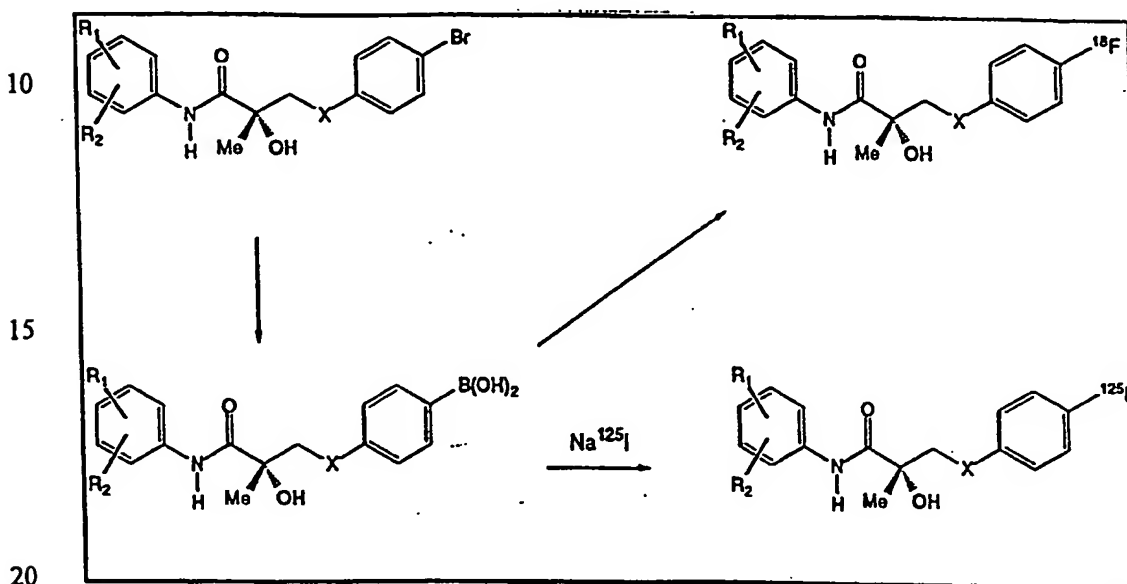25   preparation of the AR radioligands, according to the scheme below.

- 28 -
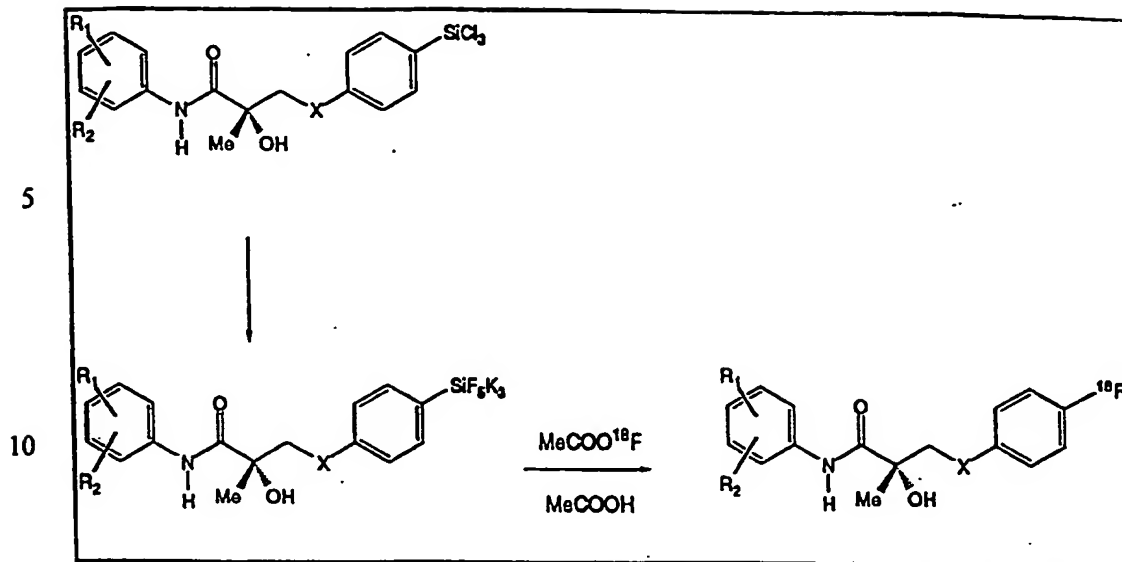
15   <u>Aryl germanes (Ar-GeR$_2$) in preparation of aromatic</u>
            <u>radiohalogenated AR ligands</u>

            Aryl germanes are chemically stable and low-toxic intermediates of

aromatic radioiodination and radiobromination (Kabalka, "The Synthesis of Radiolabeled

20   Compounds Via Organometallic Intermediates," <u>Tetrahedron</u>, 45:6601-21 (1989)). Their

use for synthesis of radiohalogenated AR ligands is shown below.

- 29 -
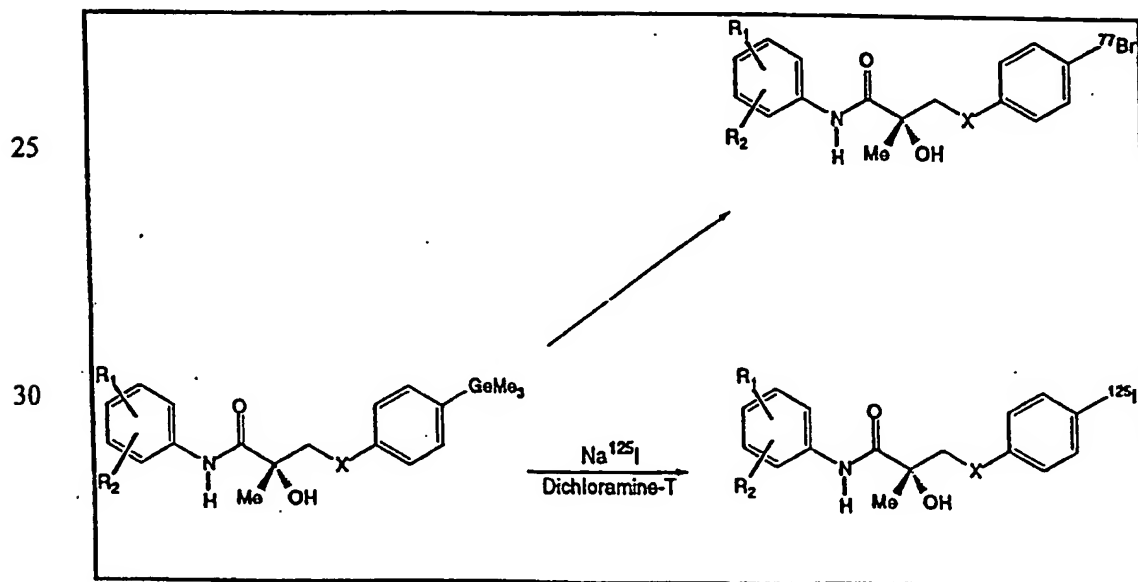
Aryl mercuriates in preparation of aromatic radiohalogenated AR ligands

A tributyltin precursor is used as shown below for the synthesis of
radioiodinated AR ligands. The advantage of these precursors in the incorporation of
radiohalogens are their easy preparation and chemical stability. However, they are highly
toxic (Kabalka, "The Synthesis of Radiolabeled Compounds Via Organometallic
Intermediates," Tetrahedron, 45:6601-21 (1989)).
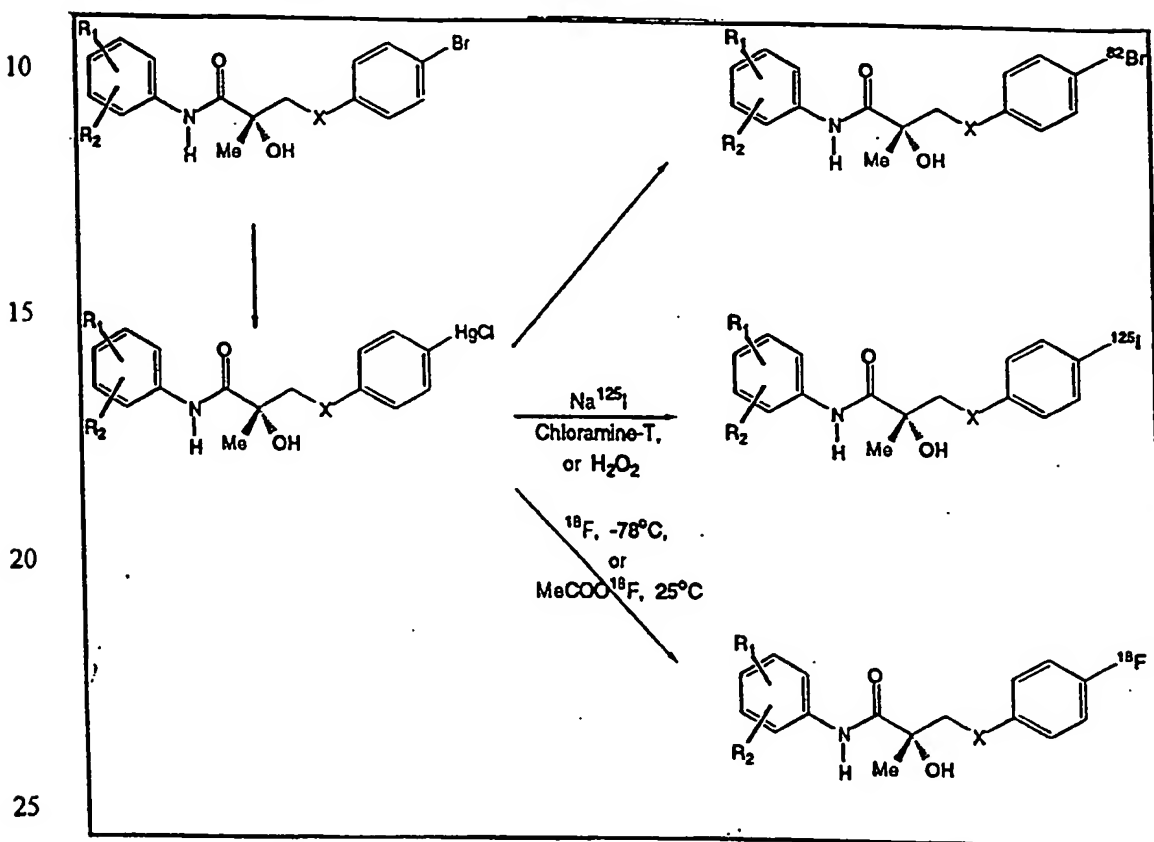
Aryl thallates Ar-Tl(OCOCF3)2 in preparation of aromatic radiohalogenated AR ligands

Aryl thallates can be prepared by predominantly *para*-substitution in an
aromatic ring (Kabalka, "The Synthesis of Radiolabeled Compounds Via Organometallic
Intermediates," Tetrahedron, 45:6601-21 (1989)). In the case of an aromatic ring
containing strong electron withdrawing substituents, thallation can be achieved in the

meta-position. As shown below, the aryl thallates may be used to produce radiolabeled AR ligands.

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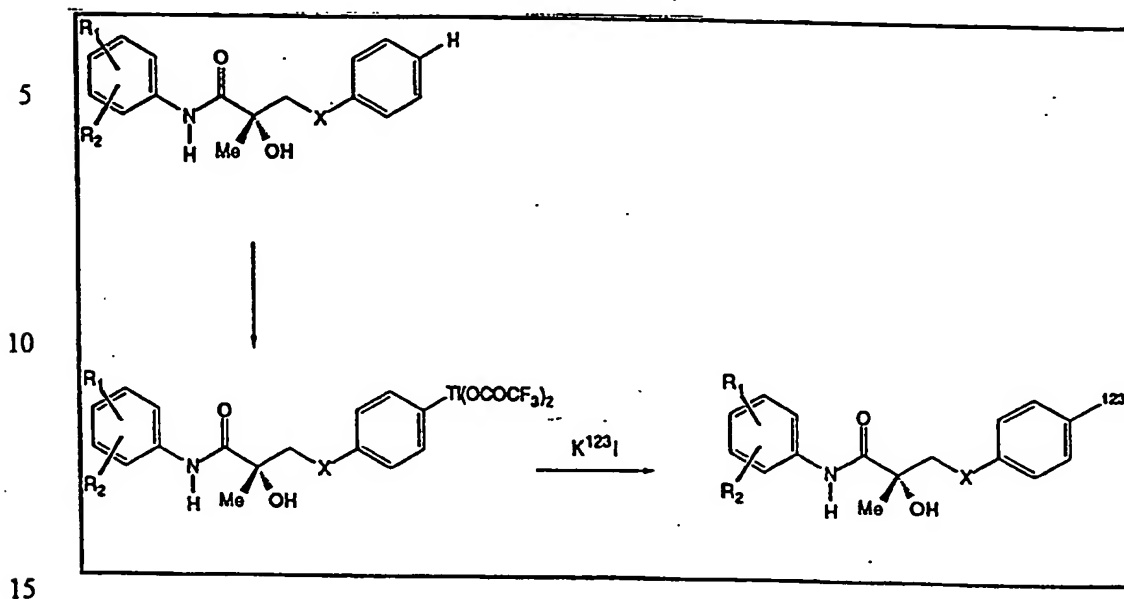
Arylthallium difluorides, shown below in the presence of boron trifluorides, can be decomposed to radiofluorides according to Taylor et al., J. Org. Chem., 42:362 (1977).

20

25
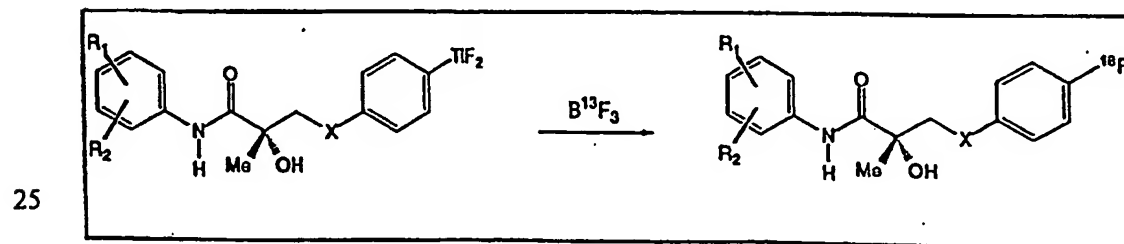
Direct halogenation

30　　　　　Direct fluorination has been done in the case of 4-borono-phenylalanine with $^{18}F_2$, or $MeCOO^{18}F$ as a source of fluorine (Ishiwata et al., "Synthesis and Radiation Dosimetry of 4-Borono-2-[18]fluoro-D,L-phenylalanine: A Target Compound for PET and Boron Neutron Capture Therapy," Int. J. Rad. Appl. Instrum. [A], 42:325-28

(1991); Reddy et al., "4-Borono-2-," J. Labeled Comp. Radiopharm., 37:599 (1995)). Similarly, fluorination of an amino precursor can be carried out.
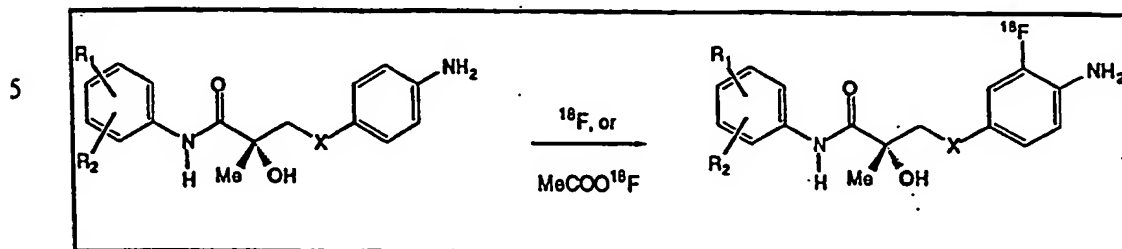
10   Direct iodination with TlCl$_3$ is described in Resek, "Photoaffinity Labeling," J. Biol. Chem., 263:14410-16 (1988).

Triflates, mesylates and tosylates

15   Triflates and tosylates are used for nucleophilic displacement leading to a radiohalogenated product. Katzenellenbogen used triflates as precursors for [18F]-fluorinated steroidal androgen receptor ligands in the synthesis of steroidal AR ligands (Liu et al., "Synthesis of High-Affinity Fluorine-Substituted Ligands for the Androgen Receptor: Potential Agents for Imaging Prostatic Cancer by Positron Emission

20   Tomography," J. Med. Chem., 35:2113-29 (1992); Liu et al., "Fluorine-18-labeled Androgens: Radiochemical Synthesis and Tissue Distribution Studies on Six Fluorine-Substituted Androgens, Potential Imaging Agents for Prostatic Cancer," J. Nucl. Med., 33:724-34 (1992); Pomper et al., "11.beta.-Methoxy-, 11.beta.-Ethyl, and 17.alpha.-Ethynyl-substituted 16.alpha-Fluoroestradiols: Receptor-Based Imaging Agents with

25   Enhanced Uptake Efficiency and Selectivity," J. Med. Chem., 33:3145-55 (1990)). Tetrabutylammonium [18F]fluoride was the carrier for the radiohalogen. Thus, displacement of triflates with K18F described in Zhang et al., "Synthesis and Evaluation of Two," J. Med. Chem., 39:5110-18 (1996) was performed according to [Kiesewetter, 1989 #4735]. Similarly, a precursor can also be easily prepared from iodide using

30   commercially available silver triflate (e.g., silver trifluoromethanesulfonate, Aldrich Chemical Co., Milwaukee, Wisconsin). (See, also the procedure for mild etherification of alcohols with primary alkyl halides (Tetrahedr. Lett., 35:8111 (1994); Tetrahedr. Lett., 36:719 (1995)).
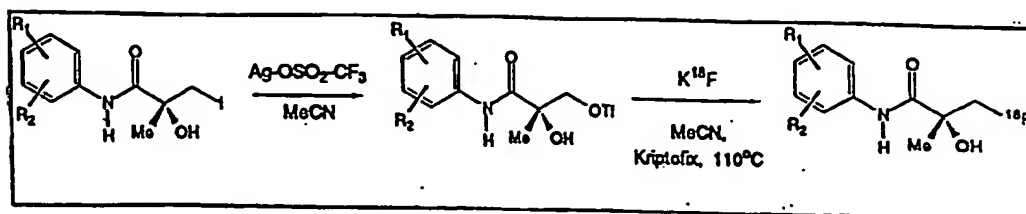
The use of mesylates as a precursor for fluorination is described in
Kiesewetter et al., "Synthesis and Biological Properties of," J. Med. Chem., 38:1711-19
(1995). The authors used Me_4NHF_2 as a source of $[^{19}F]$-fluorine. Methylates can be
easily prepared from iodide (shown below) using commercially available silver mesylate
(e.g., methanesulfonic acid silver salt, Acros, Coral Gables, Florida).
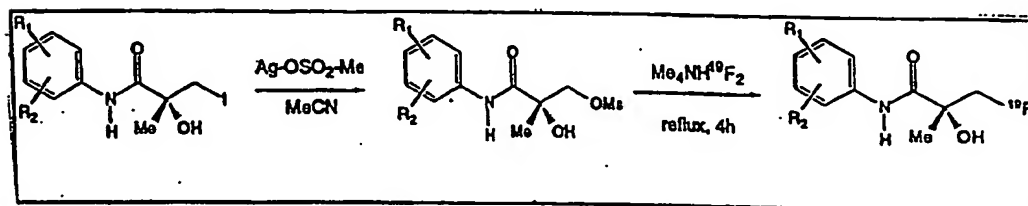
Trifluoroacetates

Aliphatic trifluoroacetates are used for fluorination of estradiols with
Bu_4NF as a source of fluorine (Pomper et al., "11.beta.-Methoxy-, 11.beta.-Ethyl, and
17.alpha.-Ethynyl-substituted 16.alpha-Fluoroestradiols: Receptor-Based Imaging
Agents with Enhanced Uptake Efficiency and Selectivity," J. Med. Chem., 33:3145-55
(1990)). As similar method can be applied to AR ligands.
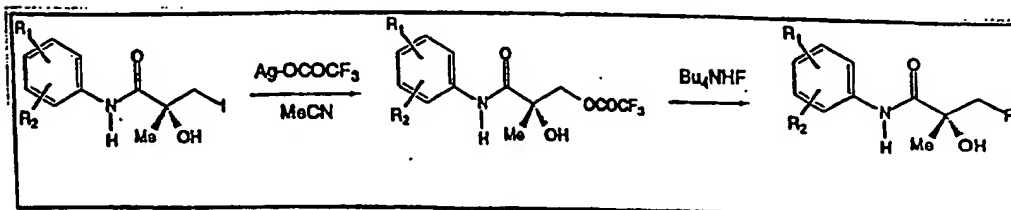
<u>Epoxides</u>

Opening of epoxides can be used for radiohalogenation as shown below.

5
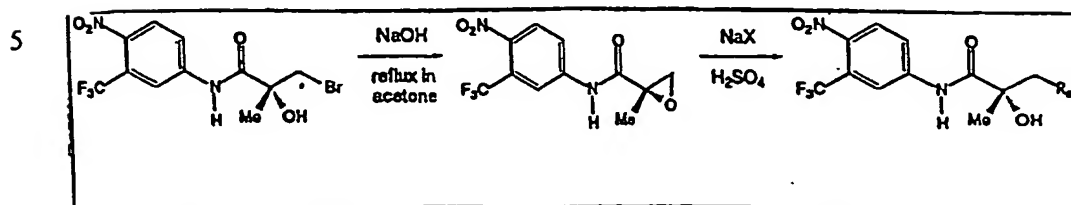
10   where R_8 is a phenyl substituent as set forth above, preferably a radiohalide.

<u>Cyclic sulfates</u>

Katzenellenbogen used cyclic sulfates as precursors for radiofluorination

15   of steroidal androgen receptor ligands metribolone (R1881) (Liu et al., "Fluorine-18-
Labeled Androgens: Radiochemical Synthesis and Tissue Distribution Studies on Six
Fluorine-Substituted Androgens, Potential Imaging Agents for Prostatic Cancer," <u>J. Nucl.</u>
<u>Med.</u>, 33:724-34 (1992)), in the synthesis of steroidal AR ligands (Liu et al., "Synthesis
of High-Affinity Fluorine-Substituted Ligands for the Androgen Receptor. Potential

20   Agents for Imaging Prostatic Cancer by Positron Emission Tomography," <u>J. Med. Chem.</u>,
35:2113-29 (1992)). Tetrabutylammonium [^{18}F]fluroide was the carrier for the
radiohalogen. Synthesis of cyclic sulfates is described in Liu et al., "Synthesis of High-
Affinity Fluorine-Substituted Ligands for the Androgen Receptor. Potential Agents for
Imaging Prostatic Cancer by Positron Emission Tomography," <u>J. Med. Chem.</u>, 35:2113-

25   29 (1992). Cyclic sulfates are useful precursors for radiohalogenation to AR ligands.

- 34 -
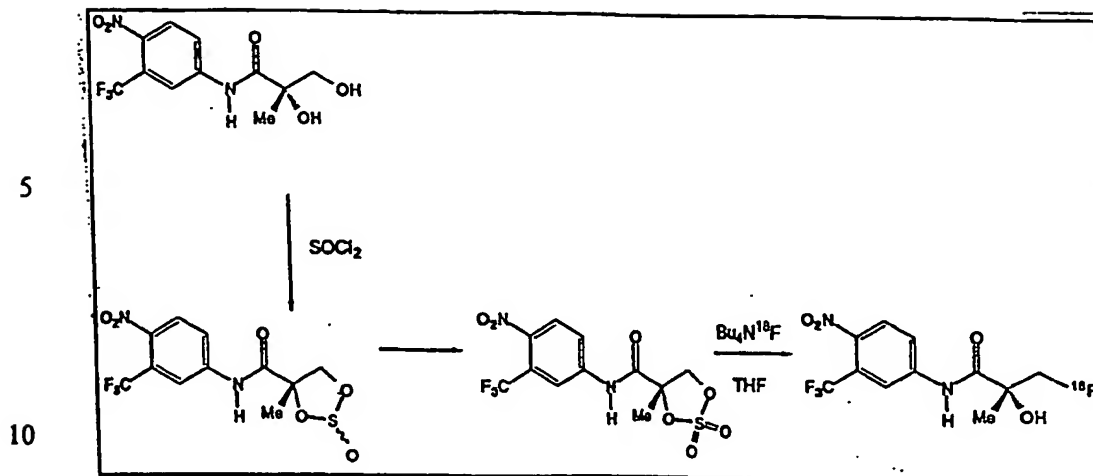
Exchange of alcohols for halogens

An iodinated compound can be prepared following the procedure of Hoyte et al., ("Synthesis and Evaluation of 7 alpha-iodo-5 alpha-dihydrotestosterone as a Potential Radioligand for Androgen Receptor," J. Med. Chem., 37(8):1224-30 (1994) as shown below:
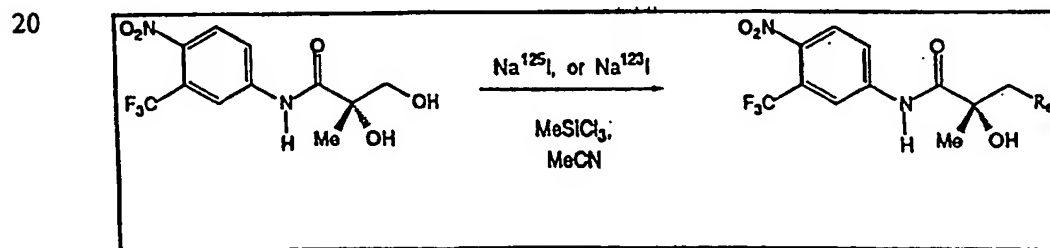
where R_8 is a phenyl substituent as set forth above, preferably a radiohalide.

Radiolabeled compounds can be prepared using the scheme shown below and described in Berridge et al., "Design and Synthesis of 18F-labeled Neurotoxic Analogs of MPTP," J. Med. Chem., 36:1284-90 (1993); Berridge, "Chemistry of Fluorine-18 Radiopharmaceuticals," Appl. Radiat. Isot., 37:685-93 (1986)); Liu et al., "Synthesis of High-Affinity Fluorine-Substituted Ligands for the Androgen Receptor.

Potential Agents for Imaging Prostatic Cancer by positron emission tomography," J. Med. Chem., 35:2113-29 (1992).
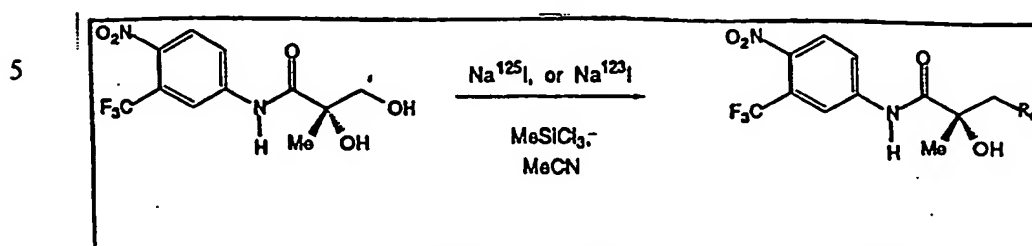
where R_8 is a phenyl substituent as set forth above, preferably a radiohalide.

Exchange of halogens

The radiolabeled compounds of the present invention can be produced using the scheme shown below.
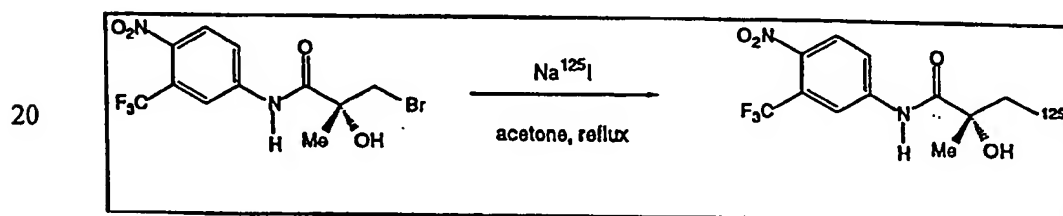
As shown below, the radiolabeled compound can be produced from alkyl bromides (Kiesewetter et al., "Synthesis and Biological Properties of," J. Med. Chem., 38:1711-19 (1995); Berridge et al., "Design and Synthesis of 18F-Labeled Neurotoxic Analogs of MPTP," J. Med. Chem., 36:1284-90 (1993). It is possible to displace aliphatic bromide for fluoride with potassium [^{19}F]fluoride and Kriptofix (Berridge, "Chemistry of Fluorine-18 Radiopharmaceuticals," Appl. Radiat. Isot., 37:685-93 (1986); Goodman et al., "Synthesis of [18F]-N-3-Fluoropropyl-2-," J. Labeled Comp. Radiopharm., 35:432-434 (1994)).

5
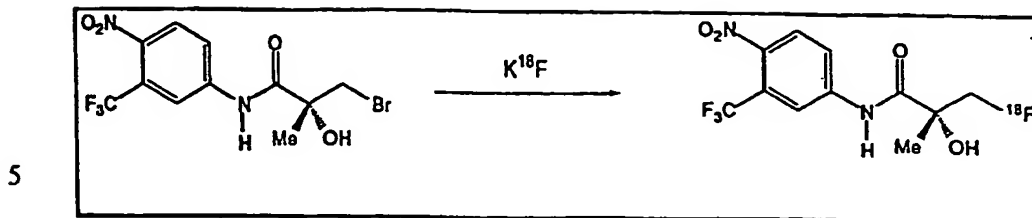
As shown below, radiolabeled compounds of the present invention can be obtained using the procedure described in Berridge et al., "Design and Synthesis of 18F-

10   Labeled Neurotoxic Analogs of MPTP," J. Med. Chem., 36:1284-90 (1993).

15
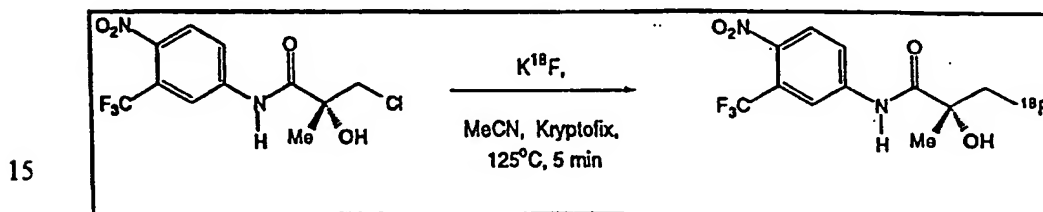
Aliphatic organoboranes in preparation of radiohalogenated
AR ligands

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As shown below, the radiolabeled compounds of the present invention can be obtained as described in Kabalka, "The Synthesis of Radiolabeled Compounds Via Organometallic Intermediates," Tetrahedron, 45:6601-21 (1989).

- 37 -
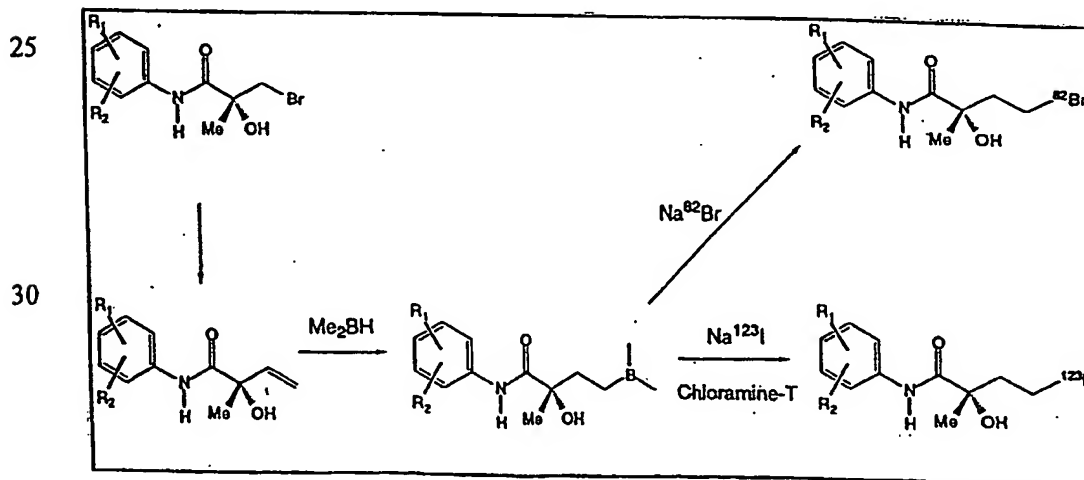
As shown in the schemes above, R_6 is a methyl group and R_5 is a hydroxy group. However, R_5 and R_6 may have any of the substituents as described above and the reaction schemes may be used to produce either the R or S isomers.

5   EXAMPLES

The following examples are provided to illustrate embodiments of the present invention, but are by no means intended to limit its scope.

10   Example 1 -   Synthesis of the R-Isomers of Iodinated Compounds

Iodinated compounds were synthesized as shown below, utilizing bromo acid as a key intermediate.

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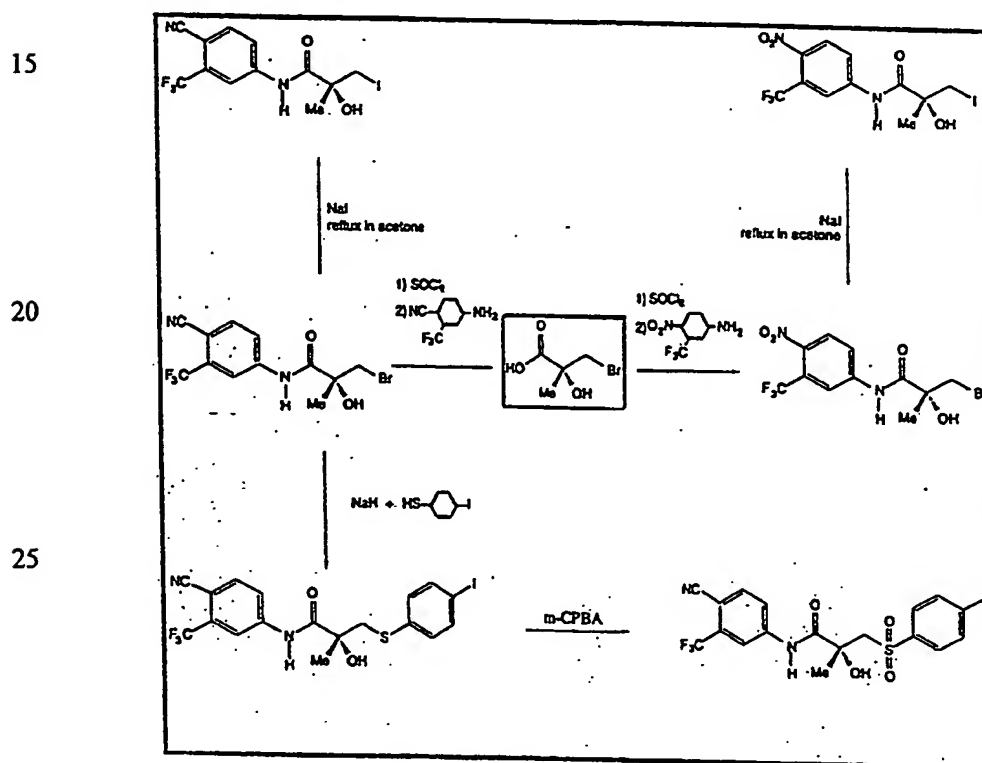
30   Aliphatic iodides and the aryl iodides were synthesized from this common intermediate. Since 4-iodothiophenol is not commercially available, it was synthesized by reduction of 4-iodobenzenesulfonyl chloride in a Sn/HCl system according to Backer et al., "Éthers Radiaires de l'Acide Tètrahioorthostannique II," Rec. Trav. Chim.,

53:1101-12 (1934). Oxidation of R-iodo-thio-Bicalutamide with m-choroperbenzoic acid gave the desired iodo analog.

Example 2 -   Synthesis of R-Isomers of Iodine Analogs of Hydroxyflutamide and Bicalutamide

Non-radioactive R-isomers were synthesized as described in the reaction scheme below.
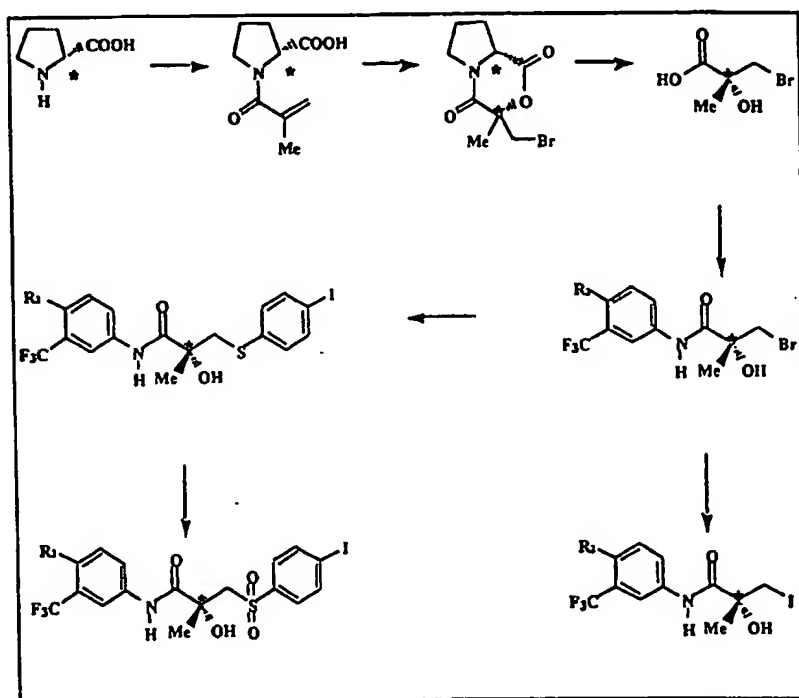
where R_2 is NO_2 or CN.

To synthesize "cold" (unlabeled) iodinated AR ligands, a reaction sequence applied for preparation of S-Bicalutamide (Tucker 1988a; Tucker 1988b) was modified by starting with R-proline as the chiral auxiliary. Thus, bromo-acid, a critical intermediate, was obtained as a pure enantiomer. R-proline was coupled with methacryloyl chloride under Schotten-Baumann conditions in aqueous acetone at 5-15°C. Terashima asymmetric bromolactonization (Jew et al., "Asymmetric Synthesis of Optically Active a,a-Substituted-a-Hydroxy Acid from a,b-Unsaturated Acids by the Novel Use of Halolactonization Reaction," Tetrahedron 35:2337-43 (1979); Nishihara et al., "Conformation of Methacryloyl-l-proline," Bull. Chem. Soc. Japan, 46:3894-5 (1973)) was used to prepare bromolactone with good yield by reaction of prolinamide

with NBS in DMF at room temperature. Hydrolysis of bromolactone in 24% HBr under reflux for 1 hour gave rise a bromo-acid with high yield. Coupling of chloroanhydride of bromo acid, obtained *in situ* by reaction of bromo acid with thionyl chloride in DMA at approximately -10°C, with 4-nitro-3-trifluoromethylaniline or 4-cyano-3-

5　　trifluoromethylaniline gave rise to anilides, where R_2 is NO_2 or CN. Reaction of the former anilides with iodothiophenol, ionized by sodium hydride, was carried out in THF at room temperature. Since 4-iodothiophenol is not commercially available, it was prepared by reduction of 4-iodobenzenesulfonyl chloride by tin in HCl solution according to Backer et al., "Éthers Radiaires de l'Acide Tètrahioorthostannique II," Rec. Trav.

10　　Chim., 53:1101-12 (1934). The product of the reaction, an iodinated AR ligand, was oxidized by peracetic acid in acetone or ethylacetate at room temperature to form another iodinated AR ligand with the substituent R_2 as described above. Aliphatic iodinated anilides were prepared using bromine/iodine exchange reaction by reflux of anilides in acetone with the presence of approximately a 20-fold excess of sodium iodide for 4 days.

15

Example 3 -　　Binding Affinity of Iodinated & Fluorinated Compounds

　　　　　　　　AR binding affinities of the synthesized ligands were determined by competitive binding in the presence of the high affinity AR ligand, ^{3}H-mibolerone ("[3]H-

20　　MIB"). In preliminary experiments, the equilibrium dissociation constant (Kd) of MIB was determined at 4°C for 18 hours by incubating increasing concentrations of ^{3}H-MIB (0.01 to 10 nM) with cytosol. The minimum concentration of ^{3}H-MIB to saturate AR sites in the cytosol preparation was 1 nM. AR binding studies for the ligands of interest were then performed under identical conditions by incubating increasing concentrations

25　　(10^{-3} nM to 10,000 nM) of each ligand with cytosol and a saturating concentration of ^{3}H-MIB (1 nM). The incubate also contained 1 μM triamcinolone to prevent interaction of ^{3}H-MIB with glucocorticoid and progesterone receptors (Carlson and Katzenellenbogen, J. Steroid Biochem. 36:549-61 (1990)). For the determination of non-specific binding, separate experiments were conducted by adding 1,000 nM MIB to the incubate. Unless

30　　otherwise indicated, all experiments were repeated three times or more. Separation of bound and free radioactivity at the end of incubation was achieved by the HAP method, as previously described (Mukherjee, et al., Xenobiotica 26:117-22 (1996)), and 0.8 mL of the ethanolic supernatant was added to 5 mL of scintillation cocktail. Radioactivity was

counted in a liquid scintillation counter (Model LS6800, Beckman Instruments Inc., Palo Alto, CA).

To determine the Kd of MIB, data were analyzed using a modified form of the Scatchard equation:

5

$$B / F = - (B / K_d) + B_{max} / K_d \qquad \text{(Equation 1)}$$

where B was the concentration of specifically-bound ^{3}H-MIB in the incubate, F was the concentration of free ^{3}H-MIB in the incubate, and B_{max} was the maximum concentration

10   of ^{3}H-MIB specifically bound to AR, which is also the concentration of available AR binding sites in the incubate. Experimental data were computer-fit to equations using fortran subroutines written for PCNONLIN (SCI Software, Lexington, KY).

For competition binding experiments, competitive displacement curves were constructed for each ligand with percent specific binding (specific binding of ^{3}H-

15   MIB at a particular ligand concentration expressed as a percentage of the specific binding of ^{3}H-MIB in the absence of ligand) on the vertical axis and ligand concentration on the horizontal axis. The ligand concentration that reduced the percentage of specific binding by 50% (IC_{50}) was determined by computer-fitting the data for each ligand to the following equation:

20

$$B = B_0 \{1 - (C / IC_{50} + C)\} \qquad \text{(Equation 2)}$$

where B_0 is the specific binding of ^{3}H-MIB in the absence of ligand, and C is the ligand concentration. Binding affinities of the ligands were then compared using the

25   equilibrium dissociation constant (Ki) of each ligand, as calculated using the equation below:

$$Ki = \{(IC_{50} * Kd)/(L + Kd)\} \qquad \text{(Equation 3)}$$

30   where Kd was the equilibrium dissociation constant of ^{3}H-MIB as determined in Equation 1, and L was the concentration of ^{3}H-MIB in the incubate (i.e., 1 nM). Low Ki values are associated with agonist activity. The relative binding affinity ("RBA") of each compound was calculated according to the equation below:

35    $$RBA = \{(IC_{50} \text{ of DHT}) / (IC_{50} \text{ of a ligand})\} * 100\% \qquad \text{(Equation 4)}$$

The structures and AR binding affinities of these compounds are shown in Figure 1 and Table 3 below. AR binding affinities for testosterone and R-bicalutamide are included for comparison. Ligands must demonstrate high selectivity and affinity for the AR in order to compete effectively for ARs *in vivo* (and thus be useful for imaging).

5   In agreement with previous studies, significantly greater AR binding affinity was observed for the R-isomers than for their respective S-isomers. Each of the S-isomers tested showed poor AR binding affinity, with K_i values greater than 100 nM. Also a higher degree of stereoselectivity was noted for the synthesized iodinated compounds, as compared to bicalutamide. The K_i values for the R-isomers of iodinated compounds

10   were two orders of magnitude lower than that of their respective S-isomers (Table 3).

Table 3

Substituents		Name	R-isomers	S-isomers
R_8	X		Ki (nM)	Ki (nM)
I	S	Compound 1	3.4	250
I	SO$_2$	Compound 2	3.1	3500
F	S	Compound 3	1.8	70
F	SO$_2$	Compound 4 (Bicalutamide)	11.0	365
Testosterone			1.1	
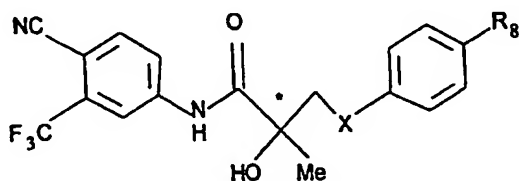
This high degree of stereoselectivity with the iodo-derivatives was unexpected, and reinforces the rationale for the use of iodinated compounds of the present invention. R-

15   isomers of Compound 2 and its un-oxidized precursor, Compound 1, demonstrated high AR binding affinity. The K_i value of the R-isomer for Compound 2 is 73% lower than that of R-Bicalutamide, and more importantly, comparable to that of the endogenous ligand testosterone. Thus, incorporation of iodine at the para-position not only allows for the introduction of alternative radionuclides for imaging, but also increases the AR

20   binding affinity in this series of compounds. This is particularly noteworthy, in that it

shows that Compounds 1 and 2 satisfy the primary requirement for *in vivo* AR-imaging (i.e., selective, high affinity AR binding).

A potential concern with these compounds may be their lower binding affinity as compared to testosterone (i.e., nanomolar concentrations of a radioligand

5   might be unable to effectively compete with endogenous testosterone for AR binding sites *in vivo*). To that end, additional structural modifications were performed to obtain ligands with sub-nanomolar affinity for the AR. As shown in Figure 1, incorporation of iodine and other halogens (e.g., fluorine, bromine) in other compounds of the present invention results in significantly higher AR binding affinity. Two of these compounds

10   (K_i=0.82±0.13 and 1.6 nM[n=2], respectively) bind the AR with greater affinity than testosterone. Further, a brominated hydroxyflutamide derivative (R-bromo-hydroxyflutamide) was synthesized which has even greater AR binding affinity (K_i=0.30 nM, n=2). In addition, a fluorinated derivative of hydroxyflutamide can be an alternative ligand for *in vivo* AR imaging. It is hypothesized that the strong electron-

15   withdrawing properties of fluorine will even further enhance the AR binding affinity and potential for *in vivo* imaging. In summary, there are several ligands with AR binding affinity similar to or greater than that of endogenous testosterone.

Carlson and Katzenellenbogen ("A Comparative Study of the Selectivity and Efficiency of Target Tissue Uptake of Five Tritium-labeled Androgens in the Rat,"

20   J. Steroid Biochem., 36:549 (1990)) showed, however, that high affinity AR binding is not sufficient by itself to conclude that an agent may be useful for *in vivo* imaging. The rate of *in vivo* metabolism and clearance is also a critical factor determining tissue selectivity and distribution, in that agents which are more slowly metabolized are able to accumulate to a greater extent in target tissues (Carlson). Nothing is known about the *in*

25   *vivo* metabolism and pharmacokinetics of compounds of the present invention. However, it is hypothesized that they are likely to undergo *in vivo* disposition like bicalutamide and flutamide. Previous studies with racemic ^{14}C-labeled bicalutamide showed that the parent compound accounts for virtually all of the radioactivity in plasma (Cockshott et al., "The Pharmacokinetics of Casodex in Laboratory Animals," Xenobiotica, 21:1347-55

30   (1991)). Thus, circulating metabolites of the compounds of the present invention should contribute little to plasma and tissue concentrations during *in vivo* studies. In summary, the high AR binding affinity, the lack of significant binding affinity of non-steroidal

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ligands for other steroid receptors and SHBG, and proposed slow *in vivo* metabolism of these compounds make them ideal candidates for selective *in vivo* imaging of AR-positive tissues and tumors.

5   Example 4 -   Synthesis of Triazene Precursor

A triazine precursor of compounds of the present invention was prepared as shown below.
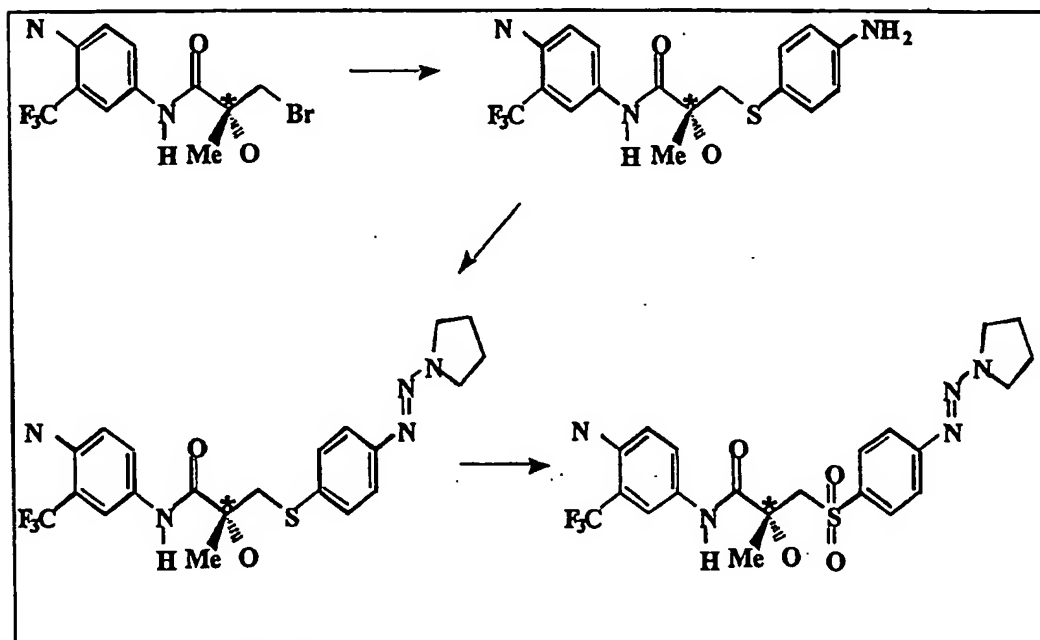
10

Synthesis of the triazine precursor was performed in three steps, starting with bromoanilide (prepared as described in Example 2). The first step involved coupling of commercially available 4-aminothiophenol, ionized by one equivalent of sodium

15   hydride, with bromoanilide in THF at room temperature to form an amino-Bicalutamide analog. Because of the electron-donating character of the amino group, ionization of 4-aminothiophenol took approximately 24 hours (unlike ionization reactions of other previously described thiophenols, which took less then 1 hour). The use of one equivalent of sodium hydride allowed any side reaction of bromoanilides with the amino-

20   portion of 4-aminothiophenol to be avoided. Preparation of the triazene compound was carried out according to general procedure described by Counsell (Van Dort et al., "Radioiodinated P-Iodoclonidine: A High-Affinity Probe For The Alpha 2-Adrenergic

- 44 -

Receptor," J. Med. Chem., 30:1241-44 (1987)). A sample of amino-Bicalutamide analog

was placed into an aqueous solution of sulfuric acid in a beaker, to which a pre-cooled

aqueous solution of NaNO$_2$ was added dropwise. The final green solution was filtered,

and pyrrolidine was added dropwise to this solution at ice-bath temperature. The yellow

5   solid product of the reaction was filtered, dried, and used for the next step without further

purification. It was oxidized to the final triazene precursor by m-chloroperbenzoic acid in

methylene chloride at room temperature.

Example 5 -   Radiochemical Synthesis and Purification of Iodinated Compounds from
10   the Triazene Precursor

Synthesis and initial purification of a radiolabeled iodo-compound of the

present invention from the triazene precursor was carried out as shown below.
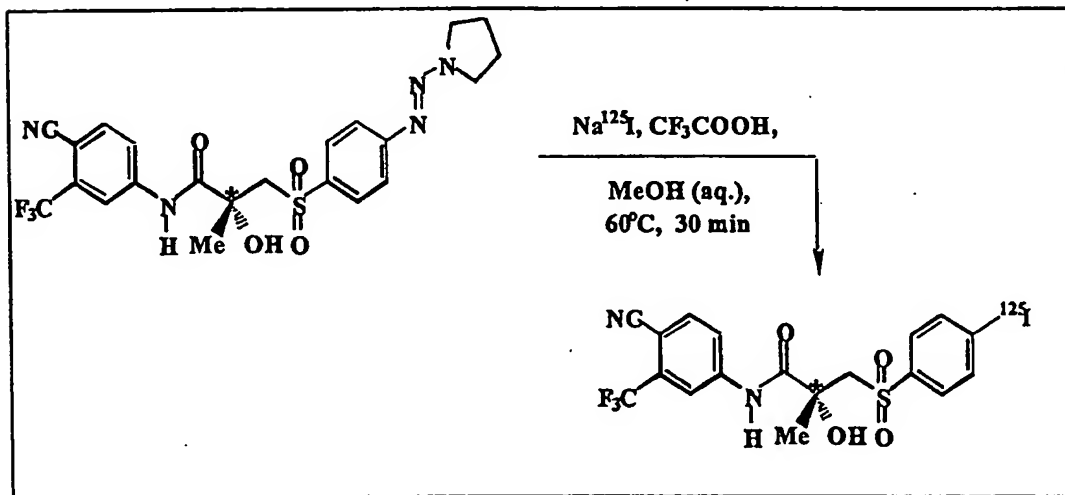
15

A methanolic reaction mixture containing trifluoroacetic acid, a 100-fold

molar excess of the triazene precursor and sodium iodide (^{125}I) were heated at 60°C for

30 minutes and then neutralized with aqueous sodium hydroxide. The reaction mixture

20   was cooled to room temperature, and immediately mixed with 5 ml of chloroform. The

chloroform extract was evaporated to dryness under a gentle stream of nitrogen, re-

dissolved in mobile phase, and then immediately injected to the HPLC system.

Separation was achieved using 35% aqueous acetonitrile as a mobile phase and a reversed

phase column (Novapak C18, Waters Chromatograph, Milford, MA). As shown in

Figure 2, the majority (>88%) of ^{125}I-labeled product eluted with a retention time of

79 minutes, corresponding exactly with the retention time of previously injected non-

radiolabeled compound standards. The determination of specific activity and

radiochemical purity of the final product is being investigated. The reactions using ^{123}I

5   sodium iodide were also successful.

Example 6 -   Synthesis of Trimethyltin Precursor

A trimethyltin precursor of compounds of the present invention was

10   prepared as below.
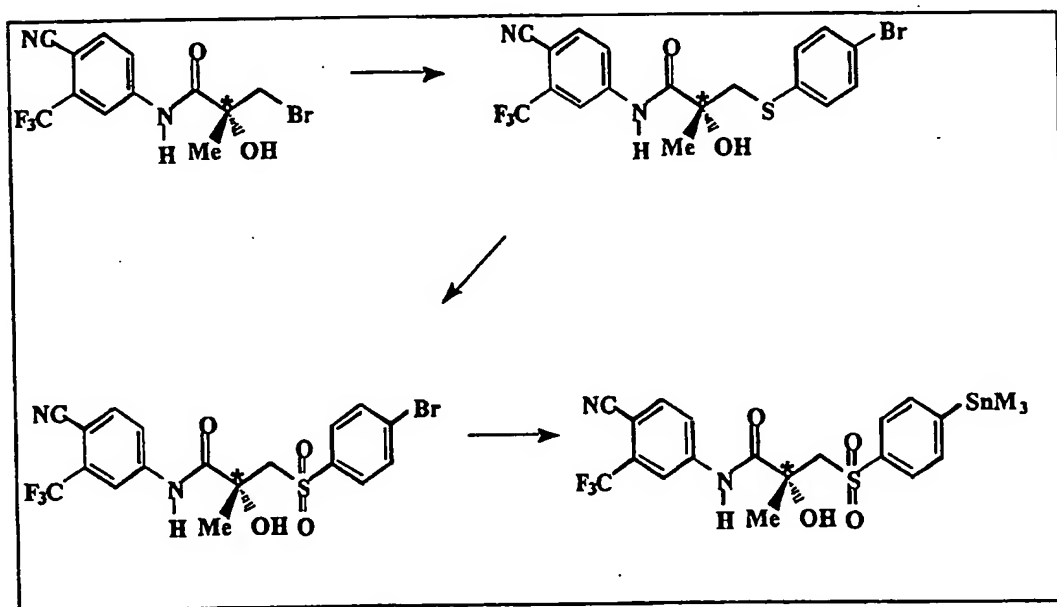
Synthesis of trimethyltin precursor was performed in three steps, starting with

15   bromoanilide (prepared as described in Example 2). The first step involved coupling of

commercially available 4-bromothiophenol, ionized by sodium hydride, with

bromoanilide in THF at room temperature. The product of the reaction was oxidized to

the bromo-analog of Bicalutamide by peracetic acid in acetone or ethylacetate at room

temperature. Reflux of the formed product in toluene with hexamethylditin Sn2Me6 and

20   Pd(PPh3)4 in the presence of triethylamine for 1 hour gave rise to the trimethyltin

precursor, which was purified by flash chromatography.

Example 7 -   Radiochemical Synthesis and Purification of Iodinated Compounds from
the Trimethyltin Precursor

Synthesis and initial purification of a radiolabeled iodo-compound of the

5   present invention from the trimethyltin precursor was carried out as shown below..
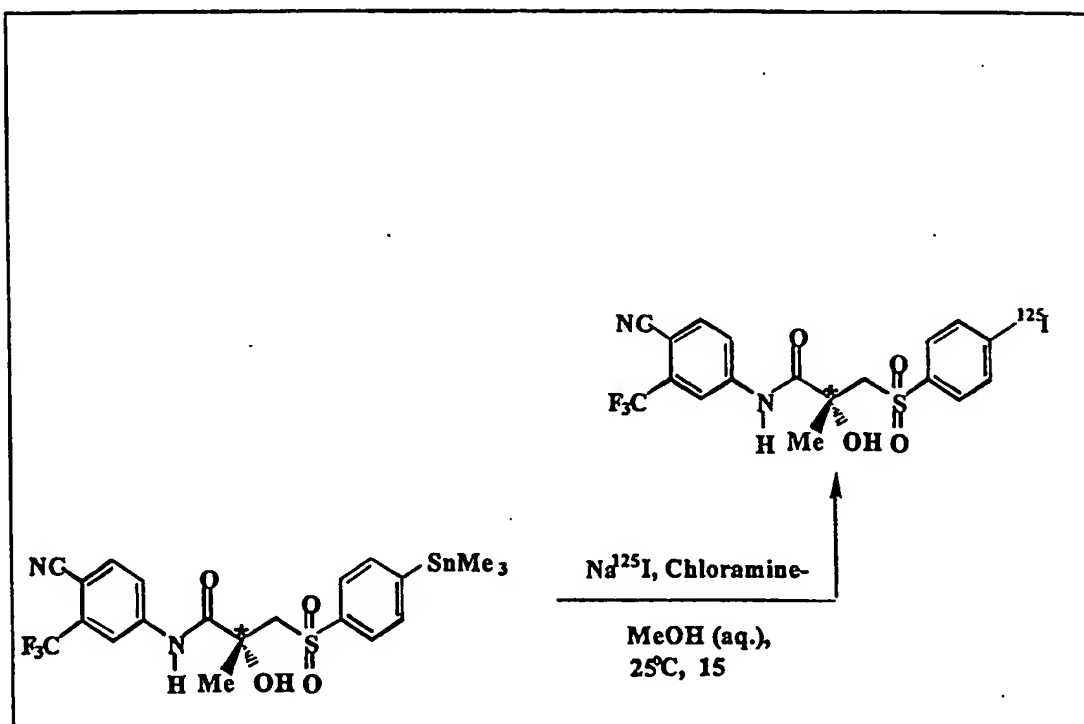

A methanolic solution of trimethyltin precursor (100 μl with approximate

10   concentration 1mg/ml) was added by syringe to a centrifuged (3000 rpm, 2 minutes)

solution of radioactive sodium iodide (Na^{125}I, 1 mCi, Amersham Corporation, Arlington

Heights, IL) in P15 microvial. Aqueous solution of Chloramine-T (Aldrich Chemical

Co., Milwaukee, IL, 50 μl with approximate concentration 2 mg/ml) was added by

syringe followed by 50 μl of 0.1N aqueous hydrochloric acid solution. The reaction

15   mixture was vortexed for 1 minute, and left at room temperature for 30 minutes. After

that, 100 μl of aqueous Na$_2$S$_2$O$_5$ solution (approximate concentration 20 mg/ml) was

injected into the reaction mixture. The reaction mixture was vortexed, and immediately

extracted 3 times with 5 ml chloroform. Chloroform layers were combined together and

dried in a flow of nitrogen. The final crude product was re-suspended into 45% aqueous

20   acetonitrile, and purified by injection into an HPLC system consisting of a Novapak C$_{18}$

reversed phase column and an on line UV detector. Fractions were collected for 1 minute

periods from the HPLC system, and the radioactivity in each fraction was counted in a

liquid scintillation counter. The total yield of the radiolabeled R-iodo-compound was

95% with specific activity 940 Ci/mmol. The trimethyltin precursor is preferred over the

5   triazene precursor because of the higher yield.

Example 8 -   Purification of Compounds from Reaction Mixtures

Compounds of the present invention were purified from crude reaction

10   mixture using either normal or reversed-phase HPLC columns.

For example, the Compound formed in Example 5 is easily and quickly

separated from its triazene precursor using an aqueous mobile phase containing 35%

acetonitrile and 25 mM phosphate buffer, pH 7.0, and a Novapak C18 reversed-phase

column (Waters Chromatography, Milford, MA) with retention times of 82 and 69

15   minutes, respectively. HPLC eluent was collected and fractions containing R-iodo-

compound were extracted with 5 ml of chloroform to yield the pure compound (>99%

chemical purity). Spiked standards showed that greater than 90% of the target compound

could be recovered from reaction mixtures using these procedures.

Normal phase chromatographic methods are also suitable for purification

20   of compounds of the present invention. Normal phase chromatography (μPorasil, 10μm

particle size columns; Waters Chromatography) allows for more rapid separation of the

reaction mixture (i.e., the R-iodo-compound of Example 5 elutes at 28 minutes in an

organic mobile phase consisting of 20% hexane in methylene chloride while the triazene

precursor is retained for 60 minutes) and avoids extraction of the compounds from the

25   aqueous mobile phase during reversed phase chromatography.

For each new ligand, the retention times of the target and precursor

compounds must first be determined in preliminary experiments by monitoring the UV

absorbance of non-radiolabeled standards. Radiolabeled target compounds are then

collected in appropriate HPLC fractions, extracted into chloroform, and evaporated to

30   dryness under nitrogen. Specific activity is then determined using quantitative HPLC.

Calibration curves are generated by injection of known standards, and the mass of

injected sample determined by comparison of its UV absorbing peak to the calibration

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curve. The activity in the eluted peak is determined by liquid scintillation counting or gamma counting. Radiochemical purity is determined using HPLC or TLC analyses. TLC separation methods are also suitable. In this instance, two-phase solvent systems are used to separate each radioligand of interest from the precursor (e.g., triazene precursor)

5   using commercially available 5x20 cm silica plates. Previous studies have shown that bicalutamide can be separated from its polar metabolites using mixtures of ethyl acetate/acetic acid (97/3 v/v) as the solvent system (Boyle, G.W., et al., "Metabolism of Casodex in Laboratory Animals," Xenobiotica 23:781-798 (1993)). To the extent they have not already been identified, TLC methods based on Boyle et al. ("Metabolism of

10   Casodex in Laboratory Animals," Xenobiotica 23:781-798 (1993) will allow for adaptation to new compounds. Analyses are then performed on a radio-TLC scanner or by scraping and gamma/beta counting. Radiochemical purity is calculated as (CPM in the peak of interest) divided by total radioactivity observed during chromatography.

15   Example 9 -   Tissue Distribution of ^{125}I-Labeled R-Iodo-Bicalutamide Following Administration

Tissue distribution studies were performed in castrated, male Copenhagen rats. A cannula was placed in the jugular vein of the rat at the time of castration. A bolus

20   dose of 100 µCi of the radiolabeled R-iodo-compound (prepared in Examples 5 and 7), in 50% ethanol in saline, was injected via the jugular vein. Rats (n=3 at each time point) were sacrificed at 0.5, 1, 2, 3, 5, 7, and 10 day time points. Organs were harvested, blotted dry, and stored at -20°C until analysis. Plasma, liver, kidney, lungs, heart, muscle, fat, prostate, seminal vesicle, epididymis, pancreas, spleen, brain, and thyroid

25   were collected. Organs were weighed, minced, and homogenized in a Pro 200 homogenizer (Pro Scientific, Monroe, CT). Homogenates were spiked with an internal standard, R-bicalutamide, and extracted with ethyl acetate, followed by centrifugation for 20 minutes at 3000. An aliquot of supernatant was dried under nitrogen, and the residue re-suspended in a mixture of 50% ethanol and n-hexane and then vortexed. Next the

30   tubes were centrifuged at 3000 rpm for 5 minutes, and the supernatant was discarded. Sodium phosphate buffer (25 mM, pH 7.0) and chloroform were added to the tubes, followed by vortexing. The tubes were centrifuged at 3000 rpm for 5 minutes, and the supernatant discarded. The chloroform layer was dried under nitrogen, and the residue re-suspended in mobile phase. An aliquot (140 µL) was injected into an HPLC system,
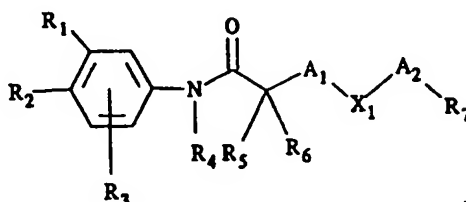
which consisted of a Waters Model 510 solvent pump (Waters, Milford, MA), a WISP 710B autoinjector (Waters), a Waters Model 480 UV detector, and a Radiomatic FLO-ONE/ℑ radioactive flow detector (Packard Instrument Company, Downers Grove, IL). Standard curves for the quantitation of the radiolabeled R-iodo-compound were

5   constructed using blank liver and lung tissue homogenates. Total radioactivity in homogenates was quantitated in a Beckman LS6800 liquid scintillation counter.

Distribution of the radiolabeled R-iodo-compound into organs was proportional to blood flow to each organ. Concentrations in liver were very similar to plasma concentrations over the entire range of time points. Distribution into lungs,

10   kidney, and heart, which have similar blood flows, was high. Distribution into prostate, seminal vesicle, and epididymis was lower due to lower blood flow to these organs. Concentrations in liver, lung, kidney, heart, muscle, spleen and brain declined in parallel with plasma concentrations, indicating the absence of selective retention in these tissues. Concentration-time profiles in prostate, seminal vesicle and epididymis, which are rich in

15   AR, showed a plateau at 3 to 5 days, suggesting selective retention in these tissues. This was further confirmed using log tissue concentration versus log plasma concentration plots, as shown in Figure 3. While other organs showed a slope close to 1.0, slopes for prostate, seminal vesicle and epididymis were significantly lower, indicating that concentrations in these tissues did not decline in parallel with plasma concentration.

20   Tissue concentration to plasma concentration ratios were similar over the entire time range for all organs except prostate, seminal vesicle and epididymis. For the prostate, the ratio increased steadily up to 5 days, when it exceeded the ratio in all other organs except the liver. These data indicate that the tissue distribution of non-steroidal androgen receptor ligands is favorable to permit non-invasive imaging of androgen receptor-

25   positive tissues, including the prostate and associated tumors.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of

30   the invention which is defined by the following claims.

WHAT IS CLAIMED:

1.   A radiolabeled non-steroidal compound having the formula:

where R_1, R_2 and R_3, are the same or different and are a radioactive or nonradioactive halogen, a nitro, a cyano, a carbamoyl, a hydrogen, a perfluoroalkyl, a haloalkylamido, an isothiocyanate, an azide, a diazocarbonyl, a substituted oxirane, or a ß-chloroethylamine;

R_4 is a hydrogen, an alkyl, or is joined to R_5;

R_5 is a radioactive or nonradioactive halogen, a hydrogen, a hydroxy, an alkyl, a haloalkyl, an alkoxy, an acyloxy, an amino, an alkylamino, a thio, a thioalkyl, or is joined to R_4;

R_6 is a hydrogen, an alkyl, a halogen, or a haloalkyl;

A_1 and A_2 are the same or different, and each is a direct link or an alkylene;

X_1 is a radioactive or nonradioactive halogen, an oxygen, a sulfur, a sulphinyl, a sulphonyl, an amino, an alkylimino, or alkylene;

R_7 is a radioactive or nonradioactive halogen, a hydrogen, an alkoxy, a haloalkoxy, an acyloxy, a haloacyloxy, an aryloxy, a thioalkyl, a thioaryl, an alkylsulphinyl, an arylsulphinyl, an alkylsulphonyl, an arylsulphonyl, an amino, an alkylimino, an alkylamido group, a radioactive or nonradioactive haloalkylamido group, a phenyl optionally substituted with a radioactive or nonradioactive halogen, a nitro group, an alkyl, a radioactive or nonradioactive haloalkyl, a cyano, a hydroxyl, a carboxylic group, an amino, an alkylamino group, a dialkylamino group, a bisalkylamino group, a radioactive or nonradioactive haloalkylamino group, a radioactive or nonradioactive dihaloalkylamino group, a radioactive or nonradioactive bishaloalkylamino group, an acylamido group, a diacylamido group, an alkylacylamido group, a radioactive or

nonradioactive haloacylamido group, a radioactive or nonradioactive bis(haloacyl)imido group, or a radioactive or nonradioactive alkylhaloacylamido group.

2.   A radiolabeled non-steroidal compound according to claim 1, wherein the compound is an androgen receptor ligand.

3.   A radiolabeled non-steroidal compound according to claim 2, wherein the compound is an androgen receptor antagonist.

4.   A radiolabeled non-steroidal compound according to claim 2, wherein the compound is an androgen receptor agonist.

5.   A radiolabeled non-steroidal compound according to claim 1, wherein the compound is a substantially pure R-enantiomer.

6.   A radiolabeled non-steroidal compound according to claim 1, wherein

R_1 is CF_3,

R_2 is CN or NO_2,

R_3 is H,

R_4 is H and R_5 is OH, or R_4 and R_5 are joined together as SCO,

R_6 is CH_3 or CF_3,

A_1 is an alkylene or a direct link,

A_2 is a direct link,

X_1 is CH_2, S, or SO_2, and

R_7 is a radioactive or nonradioactive halogen, or a phenyl optionally substituted with a radioactive or nonradioactive halogen, a nitro group, an alkyl, a radioactive or nonradioactive haloalkyl, a cyano, a hydroxyl, a carboxylic group, an amino, an alkylamino group, a dialkylamino group, a bisalkylamino group, a radioactive or nonradioactive haloalkylamino group, a radioactive or nonradioactive dihaloalkylamino group, a radioactive or nonradioactive bishaloalkylamino group, an acylamido group, a diacylamido group, an alkylacylamido group, a radioactive or

nonradioactive haloacylamido group, a radioactive or nonradioactive bis(haloacyl)imido group, or a radioactive or nonradioactive alkylhaloacylamido group.

7.   A radiolabeled non-steroidal compound according to claim 6, wherein
R_2 is CN or NO_2,
R_4 is H,
R_5 is OH,
A_1 is a direct link,
X_1 is CH_2, and
R_7 is a radioactive halogen.

8.   A radiolabeled non-steroidal compound according to claim 6, wherein
R_2 is CN,
R_4 is H,
R_5 is OH,
A_1 is an alkylene,
X_1 is S or SO_2, and
R_7 is a phenyl group substituted with a radioactive halogen.

9.   A radiolabeled non-steroidal compound according to claim 6, wherein
R_2 is CN,
R_4 and R_5 are joined together as SCO,
A_1 is an alkylene,
X_1 is S or SO_2, and
R_7 is a phenyl group substituted with a radioactive halogen.

10.   A composition comprising:
a radiolabeled non-steroidal compound and a pharmaceutically acceptable carrier;
wherein the radiolabeled non-steroidal compound has the formula:

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$$R_1, R_2 \text{ benzene ring structure with } N-C(=O)-A_1-X_1-A_2-R_7 \text{ substituents, } R_4 R_5 R_6, R_3$$

where R_1, R_2 and R_3, are the same or different and are a radioactive or nonradioactive halogen, a nitro, a cyano, a carbamoyl, a hydrogen, a perfluoroalkyl, a haloalkylamido, an isothiocyanate, an azide, a diazocarbonyl, a substituted oxirane, or a ß-chloroethylamine;

R_4 is a hydrogen, an alkyl, or is joined to R_5;

R_5 is a radioactive or nonradioactive halogen, a hydrogen, a hydroxy, an alkyl, a haloalkyl, an alkoxy, an acyloxy, an amino, an alkylamino, a thio, a thioalkyl, or is joined to R_4;

R_6 is a hydrogen, an alkyl, a halogen, or a haloalkyl;

A_1 and A_2 are the same or different, and each is a direct link or an alkylene;

X_1 is a radioactive or nonradioactive halogen, an oxygen, a sulfur, a sulphinyl, a sulphonyl, an amino, an alkylimino, or alkylene;

R_7 is a radioactive or nonradioactive halogen, a hydrogen, an alkoxy, a haloalkoxy, an acyloxy, a haloacyloxy, an aryloxy, a thioalkyl, a thioraryl, an alkylsulphinyl, an arylsulphinyl, an alkylsulphonyl, an arylsulphonyl, an amino, an alkylimino, an alkylamido group, a radioactive or nonradioactive haloalkylamido group, a phenyl optionally substituted with a radioactive or nonradioactive halogen, a nitro group, an alkyl, a radioactive or nonradioactive haloalkyl, a cyano, a hydroxyl, a carboxylic group, an amino, an alkylamino group, a dialkylamino group, a bisalkylamino group, a radioactive or nonradioactive haloalkylamino group, a radioactive or nonradioactive dihaloalkylamino group, a radioactive or nonradioactive bishaloalkylamino group, an acylamido group, a diacylamido group, an alkylacylamido group, a radioactive or nonradioactive haloacylamido group, a radioactive or nonradioactive bis(haloacyl)imido group, or a radioactive or nonradioactive alkylhaloacylamido group.

11.   A radiolabeled non-steroidal compound according to claim 10, wherein the compound is an androgen receptor ligand.
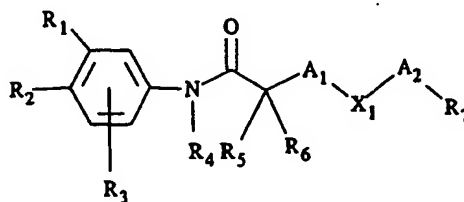
12.   A radiolabeled non-steroidal compound according to claim 11, wherein the compound is an androgen receptor antagonist.

13.   A radiolabeled non-steroidal compound according to claim 11, wherein the compound is an androgen receptor agonist.

14.   A radiolabeled non-steroidal compound according to claim 10, wherein the compound is a substantially pure R-enantiomer.

15.   A radiolabeled non-steroidal compound according to claim 10, wherein

R_1 is CF_3,

R_2 is CN or NO_2,

R_3 is H,

R_4 is H and R_5 is OH, or R_4 and R_5 are joined together as SCO,

R_6 is CH_3 or CF_3,

A_1 is an alkylene or a direct link,

A_2 is a direct link,

X_1 is CH_2, S, or SO_2, and

R_7 is a radioactive or nonradioactive halogen, or a phenyl optionally substituted with a radioactive or nonradioactive halogen, a nitro group, an alkyl, a radioactive or nonradioactive haloalkyl, a cyano, a hydroxyl, a carboxylic group, an amino, an alkylamino group, a dialkylamino group, a bisalkylamino group, a radioactive or nonradioactive haloalkylamino group, a radioactive or nonradioactive dihaloalkylamino group, a radioactive or nonradioactive bishaloalkylamino group, an acylamido group, a diacylamido group, an alkylacylamido group, a radioactive or nonradioactive haloacylamido group, a radioactive or nonradioactive bis(haloacyl)imido group, or a radioactive or nonradioactive alkylhaloacylamido group.

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16.   A method of imaging for prostate cancer or other androgen receptor containing tissues in a patient comprising:

contacting an androgen receptor with a radiolabeled non-steroidal compound under conditions effective to bind the radiolabeled non-steroidal compound to the androgen receptor and

detecting the presence of any radiolabeled non-steroidal compound bound to the androgen receptor.

17.   A method according to claim 16, wherein said contacting comprises administering the radiolabeled non-steroidal compound to a patient.

18.   A method according to claim 17, wherein said administering is oral, parenteral, or topical.

19.   A method according to claim 16, wherein said detecting is selected from a group consisting of single-photon emission computed tomography and positron emission tomography.

20.   A method according to claim 16, wherein the radiolabeled non-steroidal compound has the formula:

where R_1, R_2 and R_3, are the same or different and are a radioactive or nonradioactive halogen, a nitro, a cyano, a carbamoyl, a hydrogen, a perfluoroalkyl, a haloalkylamido, an isothiocyanate, an azide, a diazocarbonyl, a substituted oxirane, or a ß-chloroethylamine;

R_4 is a hydrogen, an alkyl, or is joined to R_5;

R₅ is a radioactive or nonradioactive halogen, a hydrogen, a hydroxy, an alkyl, a haloalkyl, an alkoxy, an acyloxy, an amino, an alkylamino, a thio, a thioalkyl, or is joined to R₄;

R₆ is a hydrogen, an alkyl, a halogen, or a haloalkyl;

A₁ and A₂ are the same or different, and each is a direct link or an alkylene;

X₁ is a radioactive or nonradioactive halogen, an oxygen, a sulfur, a sulphinyl, a sulphonyl, an amino, an alkylimino, or alkylene;

R₇ is a radioactive or nonradioactive halogen, a hydrogen, an alkoxy, a haloalkoxy, an acyloxy, a haloacyloxy, an aryloxy, a thioalkyl, a thioraryl, an alkylsulphinyl, an arylsulphinyl, an alkylsulphonyl, an arylsulphonyl, an amino, an alkylimino, an alkylamido group, a radioactive or nonradioactive haloalkylamido group, or a phenyl optionally substituted with a radioactive or nonradioactive halogen, a nitro group, an alkyl, a radioactive or nonradioactive haloalkyl, a cyano, a hydroxyl, a carboxylic group, an amino, an alkylamino group, a dialkylamino group, a bisalkylamino group, a radioactive or nonradioactive haloalkylamino group, a radioactive or nonradioactive dihaloalkylamino group, a radioactive or nonradioactive bishaloalkylamino group, an acylamido group, a diacylamido group, an alkylacylamido group, a radioactive or nonradioactive haloacylamido group, a radioactive or nonradioactive bis(haloacyl)imido group, or a radioactive or nonradioactive alkylhaloacylamido group.

21.   A method according to claim 20, wherein

R₁ is CF₃,

R₂ is CN or NO₂,

R₃ is H,

R₄ is H and R₅ is OH, or R₄ and R₅ are joined together as SCO,

R₆ is CH₃ or CF₃,
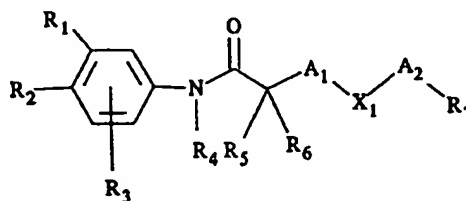
A₁ is an alkylene or a direct link,

A₂ is a direct link,

X₁ is CH₂, S, or SO₂, and

R₇ is a radioactive or nonradioactive halogen, or a phenyl optionally substituted with a radioactive or nonradioactive halogen, a nitro group, an alkyl, a

radioactive or nonradioactive haloalkyl, a cyano, a hydroxyl, a carboxylic group, an amino, an alkylamino group, a dialkylamino group, a bisalkylamino group, a radioactive or nonradioactive haloalkylamino group, a radioactive or nonradioactive dihaloalkylamino group, a radioactive or nonradioactive bishaloalkylamino group, an acylamido group, a diacylamido group, an alkylacylamido group, a radioactive or nonradioactive haloacylamido group, a radioactive or nonradioactive bis(haloacyl)imido group, or a radioactive or nonradioactive alkylhaloacylamido group.

22.   A precursor compound for producing a radiolabeled non-steroidal compound, the precursor compound having the formula:

wherein R_1, R_2 and R_3, are the same or different, and each is a hydrogen, a halogen, a nitro, a cyano, a carbamoyl, a perfluoroalkyl, a haloalkylamido, an isothiocyanate, an azide, a diazocarbonyl, a substituted oxirane, a ß-chloroethylamine, a diazonium salt, a triazene group, a tertiary alklyl group, an oxy group, an alkoxy group, a stannoalkyl group, a stannoaryl group, an unsubstituted or substituted boronic acid, an alkyl silane group, a pentaflourosilicate group, an alkylgermano group, a halomercury group, a trifluoroacetylthallate group, or a thallium difluoride group;

    R_4 is a hydrogen, an alkyl, or is joined to R_5;

    R_5 is a hydrogen, a hydroxy, an alkyl, a haloalkyl, an alkoxy, an acyloxy, an amino, an alkylamino, a thiol, a thioalkyl, a halogen, or is joined to R_4 or X_1;

    R_6 is a hydrogen, an alkyl, a halogen, or a haloalkyl;

    A_1 and A_2 are the same or different, and each is a direct link or alkylene;

    X_1 is a halogen, an oxygen, a sulfur, a sulphinyl, a sulfonyl, an amino, an alkylimino, an alkylene, or is joined to R_5 directly, through an oxirane ring, through an SO_2 group, or through an SO group;

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R$_7$ is an aryl ring, substituted at different positions with a hydrogen, a

halogen, a diazonium salt, a triazene group, a tertiary alkyl amino group, a nitro group, an

oxy group, an alkoxy group, an amino group, an alkylamino group, a stannoalkyl group, a

stannoaryl group, an unsubstituted or a substituted boronic acid, an alkyl silane group, a

pentafluorosilicate group, an alkylgermano group, a halomercury group, a trifluoroacetyl

thallate group, or a thallium difluoride group.

23.   A precursor compound according to claim 22, wherein

        R$_1$ is CF$_3$,

        R$_2$ is CN or NO$_2$,

        R$_3$ is H,

        R$_4$ is H,

        R$_5$ is OH,

        R$_6$ is CH$_3$ or CF$_3$,

        A$_1$ is an alkylene,

        A$_2$ is a direct link,

        X$_1$ is S or SO$_2$, and
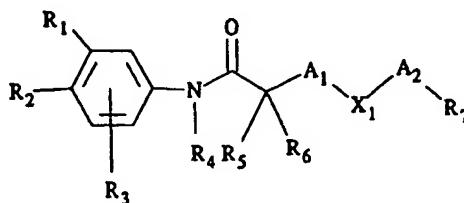
        R$_7$ is an aryl ring substituted with a triazene group, a stannoalkyl group, or

a stannoaryl group.

24.   A method of producing a radiolabeled non-steroidal compound comprising:

        providing a precursor compound according to claim 22;

        providing a radioactive compound; and

        reacting the precursor compound and the radioactive compound under

conditions effective to produce a radiolabeled non-steroidal compound having the

formula:

where R_1, R_2 and R_3, are the same or different and are a radioactive or nonradioactive halogen, a nitro, a cyano, a carbamoyl, a hydrogen, a perfluoroalkyl, a haloalkylamido, an isothiocyanate, an azide, a diazocarbonyl, a substituted oxirane, or a ß-chloroethylamine;

R_4 is a hydrogen, an alkyl, or is joined to R_5;

R_5 is a radioactive or nonradioactive halogen, a hydrogen, a hydroxy, an alkyl, a haloalkyl, an alkoxy, an acyloxy, an amino, an alkylamino, a thio, a thioalkyl, or is joined to R_4;

R_6 is a hydrogen, an alkyl, a halogen, or a haloalkyl;

A_1 and A_2 are the same or different, and each is a direct link or an alkylene;

X_1 is a radioactive or nonradioactive halogen, an oxygen, a sulfur, a sulphinyl, a sulphonyl, an amino, an alkylimino, or alkylene;

R_7 is a radioactive or nonradioactive halogen, a hydrogen, an alkoxy, a haloalkoxy, an acyloxy, a haloacyloxy, an aryloxy, a thioalkyl, a thioaryl, an alkylsulphinyl, an arylsulphinyl, an alkylsulphonyl, an arylsulphonyl, an amino, an alkylimino, an alkylamido group, a radioactive or nonradioactive haloalkylamido group, a phenyl optionally substituted with a radioactive or nonradioactive halogen, a nitro group, an alkyl, a radioactive or nonradioactive haloalkyl, a cyano, a hydroxyl, a carboxylic group, an amino, an alkylamino group, a dialkylamino group, a bisalkylamino group, a radioactive or nonradioactive haloalkylamino group, a radioactive or nonradioactive dihaloalkylamino group, a radioactive or nonradioactive bishaloalkylamino group, an acylamido group, a diacylamido group, an alkylacylamido group, a radioactive or nonradioactive haloacylamido group, a radioactive or nonradioactive bis(haloacyl)imido group, or a radioactive or nonradioactive alkylhaloacylamido group.

25.   A method according to claim 24, wherein R_7 of the precursor compound is a phenyl substituted with a triazene group or a stannoalkyl or a stannoaryl group.
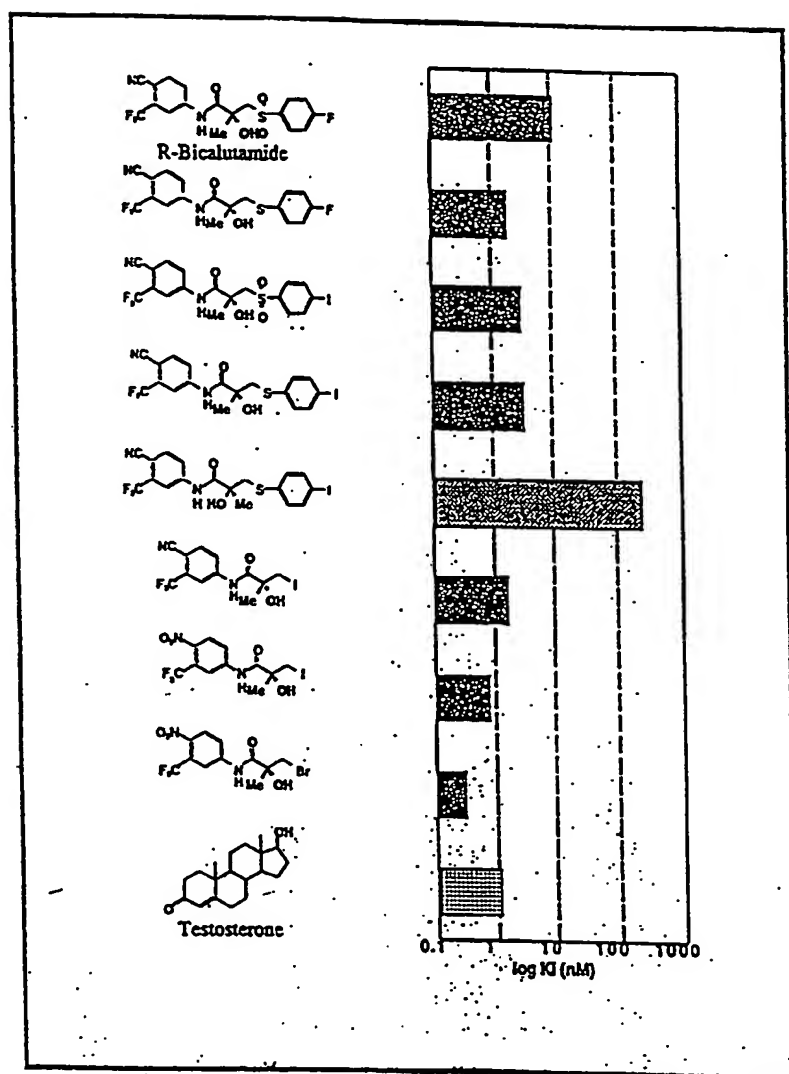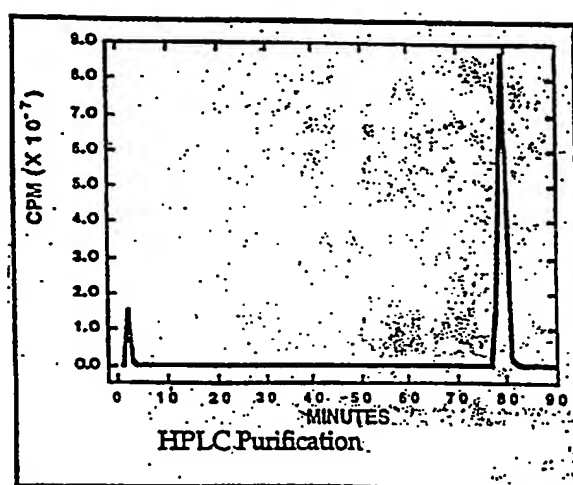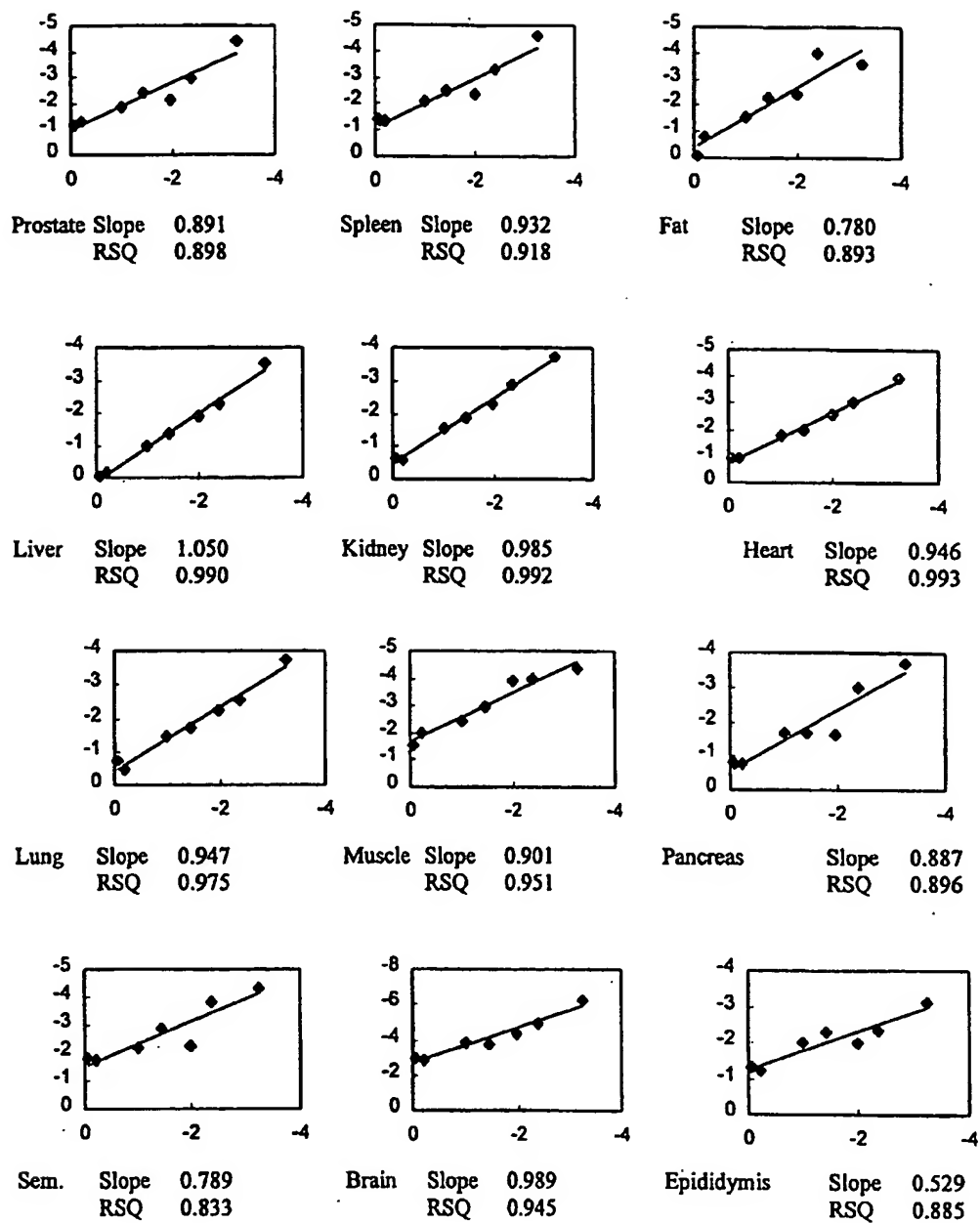
FIGURE   1

HPLC Purification.

FIGURE 2

Vertical axes represent Ln (Tissue Concentrations in %injected dose/gram of tissue)
Horizontal axes represent Ln (Plasma Concentrations in %injected dose/mL)

FIGURE 3

INTERNATIONAL SEARCH REPORT

International application No.	
	PCT/US98/11483

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 49/04, 31/615; C07C 237/00
US CL :424/1.85; 564/47; 514/166

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.85, 1.81; 564/47, 256; 514/166; 544/316

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, CAPLUS, WPIDS, MEDLINE
(search terms: androgen, prostate imaging, anilide, casodex, radioactive, bicalutamide; structure search)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,636,505 A (TUCKER) 13 January 1987, see entire document.	1-25
Y	WO 95/19770 A1 (SEPRACOR, INC.) 27 July 1995, see page 2.	1-25
Y	US 4,977,288 A (KASSIS ET AL.) 11 December 1990, see example 3 and abstract.	1-25
Y	MCKILLOP D. ET AL. Enantioselective metabolism and pharmacokinetics of Casodex in the male rat, Xenobiotica, 1995, Volume 25, Number 6, pages 623-633, see entire document.	1-25

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	
"A"	document defining the general state of the art which is not considered to be of particular relevance	
"B"	earlier document published on or after the international filing date	
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O"	document referring to an oral disclosure, use, exhibition or other means	
"P"	document published prior to the international filing date but later than the priority date claimed	

"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 AUGUST 1998	15 SEP 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Michael G. Hartley
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235

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